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BIOCHEMICAL DISTINCTIONS BETWEEN BARLEY VARIETIES [1, 2]

J. A. SHELLENBERGER and C. H. BAILEY

Division of Agricultural Biochemistry, University Farm, St. Paul, Minnesota

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Introduction

Barley is regarded by many as the most ancient cultivated grain. There is good evidence that it was a staple food in Egypt as early as 6000 B. C., and there are indications that it was more highly esteemed than wheat at that early period. Since the extended cultivation of wheat and rye displaced barley as an important human food, in more recent times, it is doubtful if barley would have retained its eminent place in agriculture were it not for its malting properties. Barley is best suited for the production of malt because the barley endosperm is covered with a husk which protects the growing acrospire from damage. In addition, its husk serves as a filtering material during the brewing process. Barley malt also contains less undesirable protein fractions than malted wheat, rye, or oats, and its amylases are more active than other cereal amylases. Therefore, when seeking distinctive properties between barley varieties those differences which become evident during malting are of primary importance.

For malting purposes certain varieties are more desirable than others, although the exact biochemical and physical reasons for the differentiation are sometimes exceedingly vague. In an effort to determine biochemical distinctions between barley varieties, an investigation into the changes in diastatic activity and starch susceptibility to diastatic action of barley, green malt, and dry malt was undertaken. Varietal dif-

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[2] Condensed from one portion of a thesis submitted to the Graduate Faculty of the University of Minnesota by J. A. Shellenberger in partial fulfillment of the requirements for the degree of Doctor of Philosophy, 1935.

TABLE I

DISTINGUISHING CHARACTERISTICS OF THE BARLEY VARIETIES

Variety	Minnesota number	Parents	Source	Agronomic characteristics
Wisconsin No. 38	529	Lion × Oderbrucker	Wisconsin Agr. Expt. Sta.	Smooth-awned, high-yielding, resistant to "Spot-blotch," moderate resistance to barley stripe, susceptible to scab and loose smut, weak-strawed.
Velvet	447	Luth × a smooth-awned select	Minnesota Agr. Expt. Sta.	Smooth-awned, susceptible to scab, stripe, and loose smut, resistant to "spot blotch" and moderately susceptible to covered smut.
Oderbrucker	528	Selection from variety brought to Canada by Ontario Agr. College from Germany	Wisconsin Agr. Expt. Sta.	Low-yielding, similar to Manchuria in type, moderately resistant to scab.
Trebi	448	Plant selection from a variety obtained from Asiatic Turkey	Bureau Plant Ind. U.S.D.A.	High-yielding feed barley, rough-awned, weak-strawed, susceptible to "Spotblotch" and covered smut, resistant to barley stripe and loose smut and moderately susceptible to scab.
Peatland	452	Selection from variety called Switzerland	Minnesota Agr. Expt. Sta.	Rough-awned, adapted to peat land, resistant to "Spotblotch," loose smut and stem smut, susceptible to stripe and moderately resistant to covered smut and scab.
Spartan	536	Cross of two Michigan barleys in 1912	Michigan Agr. Expt. Sta.	Two-rowed, smooth-awned, stiff-strawed, moderately susceptible to scab.
Not named	462	A smooth-awned selection × Manchuria	Minnesota Agr. Expt. Sta.	Smooth-awned, high-yielding, susceptible to scab.
Glabron	445	A smooth-awned selection × Manchuria	Minnesota Agr. Expt. Sta.	Smooth-awned, high-yielding, stronger strawed than Velvet. Susceptible to scab and loose smut, moderately resistant to stripe and covered smut and resistant to "Spotblotch."

ferences in the behavior of the barley samples during the steeping, germination, and drying process were closely observed as well.

Materials

The barley samples for this investigation were furnished by the Agronomy Division of the University of Minnesota from the increase plots at the University Farm and the Crookston Station at Crookston, Minnesota. The agronomic characteristics of the barley varieties studied are given in Table I. Commercially, barleys are classified in grain grading into three types, namely, two-row, six-row, and feed grade. All three types are represented in the samples selected.

Six of the eight varieties studied were grown in the same location during the years 1933, 1934, and 1935. In considering the data included in this report it should be borne in mind that the years 1933 and 1934 were not conducive to good barley production because of the extreme drouth conditions. Conditions at Crookston during the 1934 season were more favorable for barley production than at University Farm, St. Paul. The Crookston samples were included for that reason. Nevertheless, the barley used in this study is representative of what was grown in the two localities and affords an opportunity to compare certain physical and chemical properties of different barley varieties when subject to identical climatic, soil, and harvest conditions.

Composition and Properties of the Barley Varieties

The results of a partial chemical analysis, and certain other characteristics of the barley samples are given in Table II. All showed a fairly good germination value as 83% germination was the lowest figure for any of the 26 samples.

The 1000 kernel weights of the barley samples are somewhat low, indicating in many cases a small or shriveled kernel. Spartan produced the heaviest kernels as might be anticipated of a two-row variety.

The ash content is fairly uniform. The ash of the Crookston samples is slightly lower than in those from University Farm, as would be expected since the former samples are composed of plumper kernels.

The protein contents of all the samples are too high for superior malting barleys. High nitrogen content has been considered undesirable because, in general, barleys of this type were difficult to modify or "mellow" during the malting process. As a result of a statistical study, Bishop (1930) established that there was an inverse relation between the nitrogen content of the barley and the extract yield of the resulting malt. An increase of extract with increase of grain size was also conclusively demonstrated; therefore, Bishop proposed an equation

TABLE II

COMPOSITION AND PROPERTIES OF BARLEY SAMPLES

Laboratory number	Variety	Germination	Weight per bushel	1000 kernel weight	Moisture-free basis	
					Ash	Crude protein N × 6.25
		%	*Pounds*	*Grams*	%	%
	University Farm, St. Paul, Minnesota. 1933 crop					
100	Wisconsin	97	43.0	28.57	2.91	16.47
101	Velvet	97	43.1	24.21	3.07	18.30
102	Oderbrucker	99	42.1	24.71	2.88	19.74
103	Trebi	94	41.5	32.76	2.82	17.24
104	Spartan	93	48.8	41.86	2.77	18.59
105	Peatland	89	43.6	23.42	3.10	18.80
	University Farm, St. Paul, Minnesota. 1934 crop					
106	Wisconsin	88	46.5	28.12	2.80	21.40
107	Velvet	87	48.9	24.43	2.64	19.92
108	Oderbrucker	91	49.1	26.50	2.54	18.25
109	Trebi	94	41.6	26.87	2.99	20.91
110	Spartan	87	51.0	35.82	2.72	20.03
111	Peatland	83	47.3	22.02	2.66	19.83
	Crookston Station, Crookston, Minnesota. 1934 crop					
112	Wisconsin	92	51.3	31.07	2.34	14.99
113	Velvet	95	50.6	32.80	2.37	15.70
114	Oderbrucker	97	47.8	30.81	2.52	16.67
115	Trebi	89	50.5	40.17	2.21	15.20
116	Minnesota No. 462	97	51.2	30.23	2.30	14.79
117	Peatland	94	51.4	30.26	2.30	14.87
	University Farm, St. Paul, Minnesota. 1935 crop					
118	Wisconsin	94	45.4	31.87	2.66	15.37
119	Velvet	97	45.4	29.94	3.08	15.44
120	Oderbrucker	96	45.2	28.94	2.96	15.51
121	Trebi	92	45.0	37.13	2.64	15.61
122	Spartan	91	50.0	42.02	2.95	15.83
123	Peatland	96	45.4	25.08	3.00	17.07
124	Glabron	97	45.2	29.86	3.07	16.30
125	Minnesota No. 462	94	45.0	28.69	3.12	15.78

which could be used to predict the extract a barley should yield as malt from a knowledge of the variety, 1000 kernel weight, and nitrogen content of the barley.

Bishop's work was confirmed by that of Mitchell (1932) when the formula was successfully applied to 279 samples of barley and also by the work of Hind and Lancaster (1932). Recent research by Bishop

and Day (1933) has brought about a slight modification of the original formula employed by Bishop (1930). The formula now used is

$E = A - 10.5\ N = 0.20\ G$, where
E = extract in brewers' pounds per quarter on dry malt.
N = percentage of nitrogen of dry barley.
G = 1000 kernel weight of dry grain, and
A = a constant for each variety.

High yield of extract is almost invariably desirable from a malt, and since the inverse relationship of extract yield to nitrogen content has been established, the undesirability of extremely high nitrogen content of barley is evident. The problem of nitrogen in malting barely over a period of 25 years has been reviewed by Cluss (1929). He states that during this period the problem has taken an entirely new aspect because it is now realized that in addition to the quantity of nitrogen the quality must also be considered. Bishop (1930a) has worked extensively on the relation of nitrogen content to barley quality. He concluded that the soil composition and climate are more important contributing factors to the production of high nitrogen barley than are varietal differences.

Evaluation of Barley

As pointed out by Harrison (1929), and Harlan (1932) barley has two principal uses: first, for feeding farm animals and, second, for the making of malt. Malt, in turn, has a multiplicity of uses. It is necessary to know to what use a barley is to be put before it can be accurately evaluated. For example, in a feed barley nutritive value and yield are the two important factors, while for the production of pearled barley, size and shape of kernel play a major rôle, but the real critical selection of barley usually comes when malting quality is sought. Even in malting, however, there is an immense difference in the malt requirements depending on whether it is to be used for brewing, distilling, or malt extract. Generally, the requirements for brewers' malts are the most rigid; therefore, a very important test for barley varieties is to subject them to comparative malting tests.

Several systems, notably that of Wahl and Henius (1908), and Kutter (1930), have been used to evaluate barley and malt for brewing purposes by assigning a "brewing value" to the malt made from the barley. This is a numerical expression computed by adding together marks given the individual characteristics which affect brewing. Nevertheless, it is difficult as yet to predict either quality or quantity of the final product. As a help in evaluating barley, sometimes a rather simple test such as the estimation of "steeliness," or the percentage of steel

blue-gray kernels, is employed. It has long been recognized that such kernels are difficult to modify during the malting process. Ehrich and Kneip (1931) showed that when a barley sample was separated into mealy, semi-steely, and steely kernels the protein content increased in the above order. During malting it was found that the steeping period was much shorter for the mealy portion of the sample than for the steely kernels. According to Petit (1934), however, steeliness in barley is not of such great importance because it is possible, if the proper procedure is followed, to make malt from vitreous barleys. His investigation of French barleys indicated that steely kernels occur during both dry and moist growing seasons.

When the barley husk is removed as in the pearling process, it is rather easy to judge the extent of steeliness by the color of the kernel. The samples used in this study were pearled with a laboratory pearling machine. Only the variety Trebi had steely kernels.

Malting Procedure

Steeping.—In order to moisten the barley kernels sufficiently to start germination, they were immersed in water at 14° C. for 52 hours. Steeping periods appear to be influenced by (a) the character of the barley, (b) the composition of the industrial water employed, and (c) temperature. Reference will be made later to the underlying principles, and the behavior of the barley samples studied by us.

Germination.—At the termination of the steeping period the barley samples were transferred from bags into wire germination cages and placed in the previously cooled and humidified cabinet. Germination continued for about 6 days, or until the acrospire of the grain was the length of the kernel, subject to a constant change of humidified air at 16° C. The cages were revolved a quarter turn every 4 hours to insure uniform conditions for all parts of the samples. Also, aeration was thus aided, carbon dioxide removed, and matting prevented. Water was added to the germinating grain as required to promote normal acrospire growth.

Drying.—On completion of the germination period the germinated barley was rotated continuously during the drying process. The cages made one revolution every hour and were subject to the following temperatures:

24 hours at 40° C.
24 hours at 50° C.
10 hours at 85° C.

After the final drying, the malt was taken from the drier, the sprouts removed and the samples placed in air-tight containers.

Steeping researches.—A number of workers have reported researches on certain phases of the steeping process. For example, Raux (1928) discussed the changes in the barley grain during steeping which are produced by bacteria, moulds, and yeasts. Joyce (1930) studied the effect of steeping time on the quantity of extract, calculated on the original barley, and concluded that the steep period could vary considerably in normal barleys without seriously influencing the extract from the original barley. Benton (1932) gives a good review of some of the important changes occuring in barley during steeping. He contends

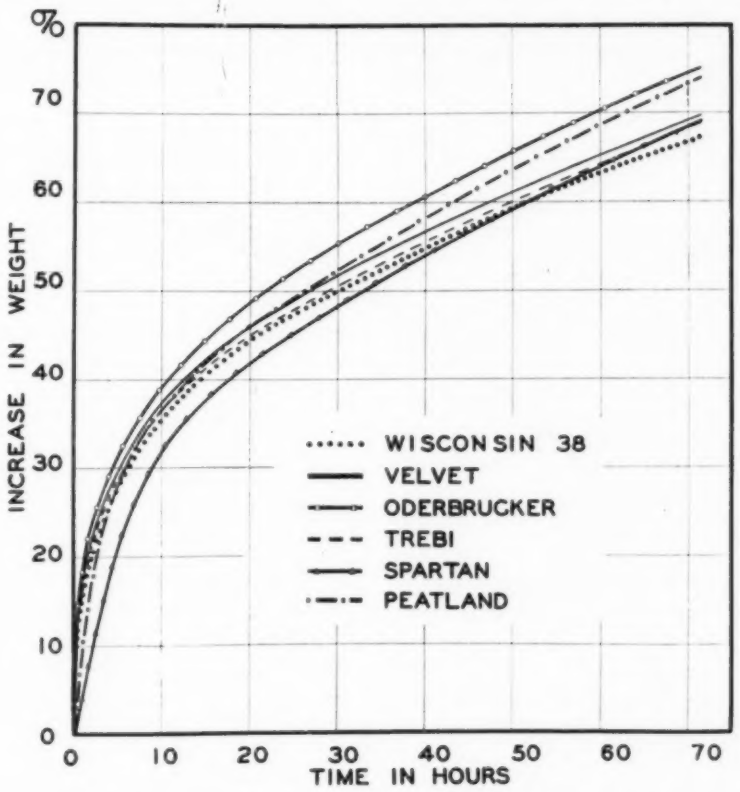

Fig. 1. Imbibition of water by barley. (1933 crop—University Farm.)

that steeping involves much more than the mere imbibition of water by grain, and that the process should be recognized as the artificial reproduction of nature's methods in the preliminary preparation of barley for growth. Brown and Worley (1912), and Brown and Tinker (1915) demonstrated that barley during steeping increases rapidly in weight at first, and more slowly as it approaches steep-ripeness. Re-

cently a centrifugal device known as the Avery Rapid Gravitmeter has tended to show "breaks" or irregularities in the normal slope of the curves. Benton (1932) calls the first break in the curve the "saturation point" corresponding to the optimum duration of the steeping process.

To obtain additional information regarding the imbibition of water by different barley varieties, the method adopted by Shellenberger and Bailey (1936) for measuring the rate of water absorption was used. Barley was steeped in wire centrifuge tubes which could be removed from the vat at intervals and centrifuged to remove the surface water.

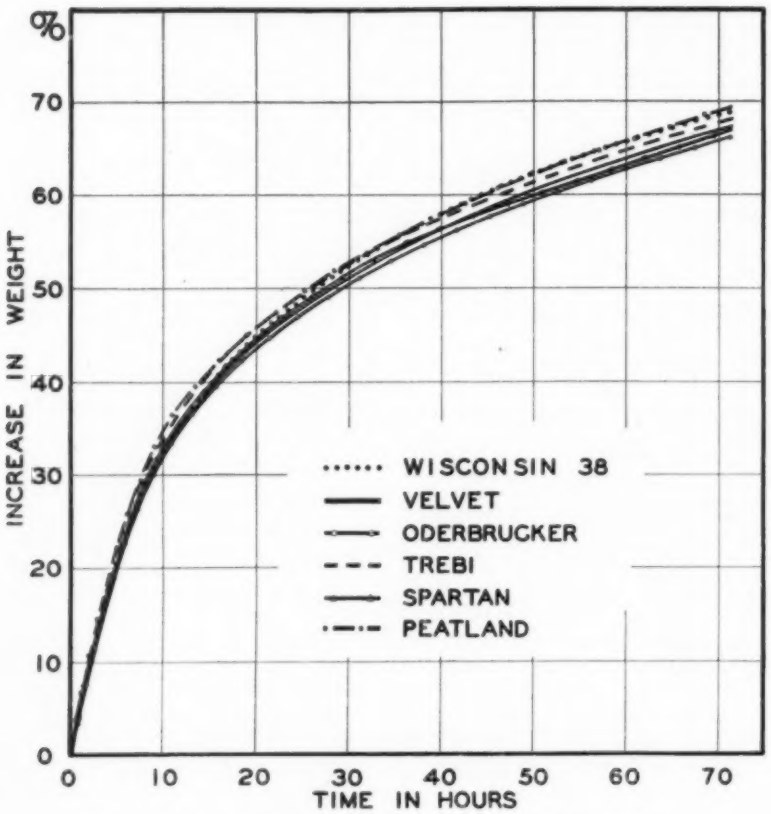

Fig. 2. Imbibition of water by barley. (1934 crop—University Farm.)

The sample was weighed and immediately returned to the steep. The percentage of increase in weight and the ratio of water to dry matter were followed in each case for a period of 70 hours. Samples from the same crop and location were steeped together and several group runs

were made in each case. The results are presented graphically in Figures 1, 2, 3, and 4. It will be noticed that no irregularities such as Benton (1932) found occur in these curves. The similarity of the curves of water imbibition for the 3 years and 2 locations for all varieties of barley is apparent, but one or two items of interest should be indicated.

The rate of imbibition of water by shriveled barley, such as that grown at University Farm in 1934 (see Figure 2), tends to be more uniform. As the kernels became more plump and rounded, the differences in the quantity of water absorbed per unit of time by different

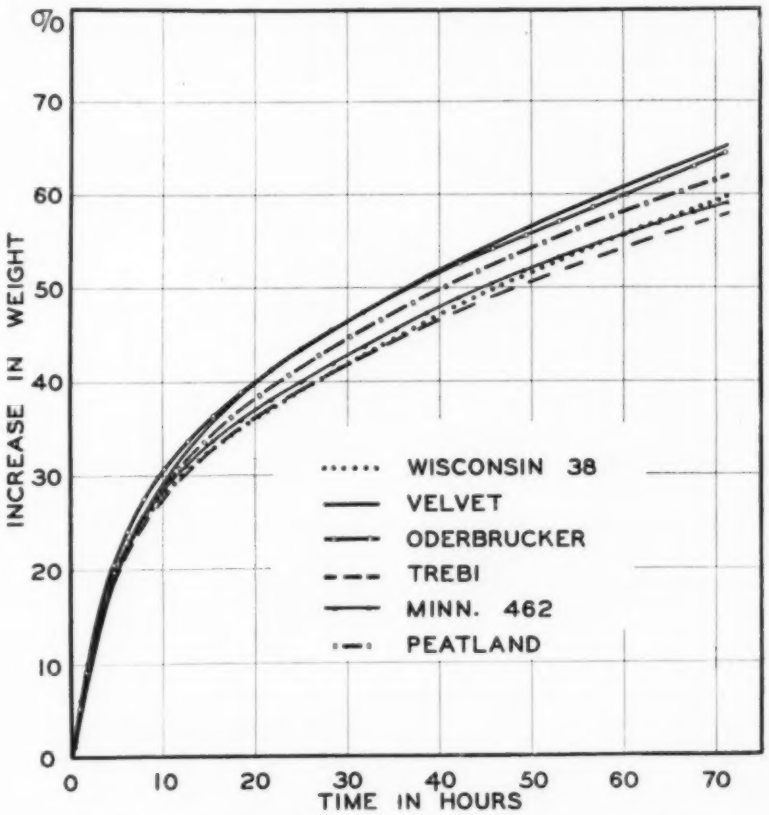

Fig. 3. Imbibition of water by barley. (1934 crop—Crookston Station.)

varieties becomes more noticeable. Inspection of Figures 1 and 4 shows that the two-row variety Spartan gave a somewhat different imbibition curve from the other varieties represented on the graphs. Unfortunately, the samples from the 1934 crop at University Farm, because of

conditions previously referred to, did not show sufficient differences to verify the conclusion that water is absorbed by two-row barley at a substantially lower initial rate than by six-row varieties.

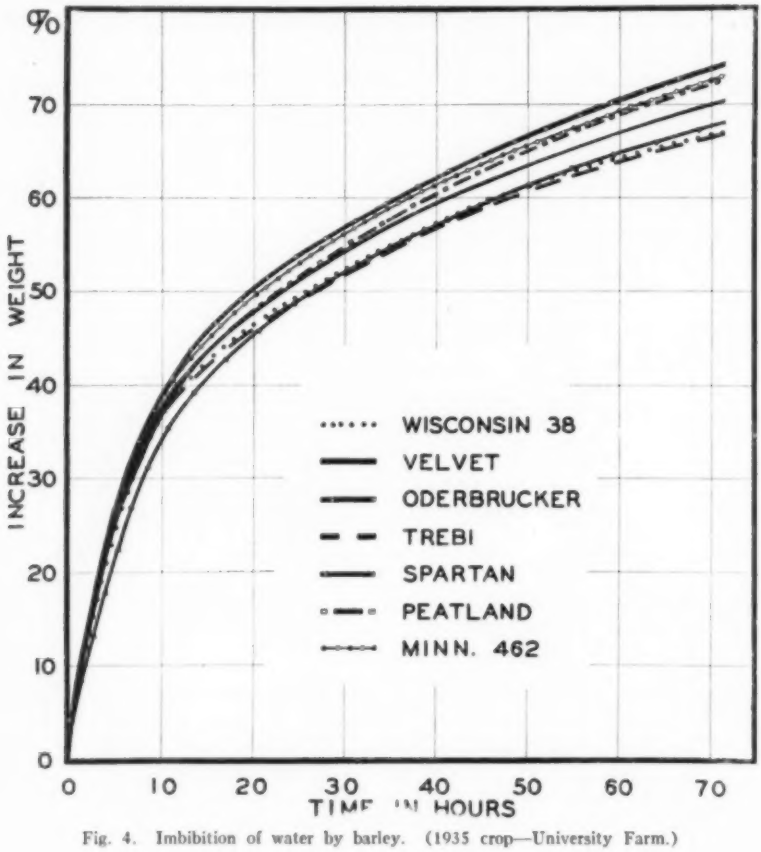

Fig. 4. Imbibition of water by barley. (1935 crop—University Farm.)

The varieties Oderbrucker and Peatland imbibe water at a more rapid rate than the other varieties studied. Trebi absorbs water at an average rate for the first few hours, but later the rate slows up rather decidedly. The barleys grown at Crookston, Minnesota, imbibed less water per unit time than did samples of the same varieties which were grown at University Farm.

The value of knowing the rate at which barley absorbs water is of importance in commercial malting because, in general, if the rate is rapid a shorter time will be required in the steep to reach optimum moisture content for germination and, consequently, the cost of producing the malt will be reduced.

Acrospire growth.—Uniformity of kernel modification is a very desirable quality in malting barley. This property is judged by the uniformity of acrospire growth in terms of the length of the germinated kernel. Germination is usually stopped when the largest number of kernels have an acrospire length of from three-quarters to the full extent of the kernel. When a large proportion of a sample is included within this range it receives a high rating on the score of uniformity of germination.

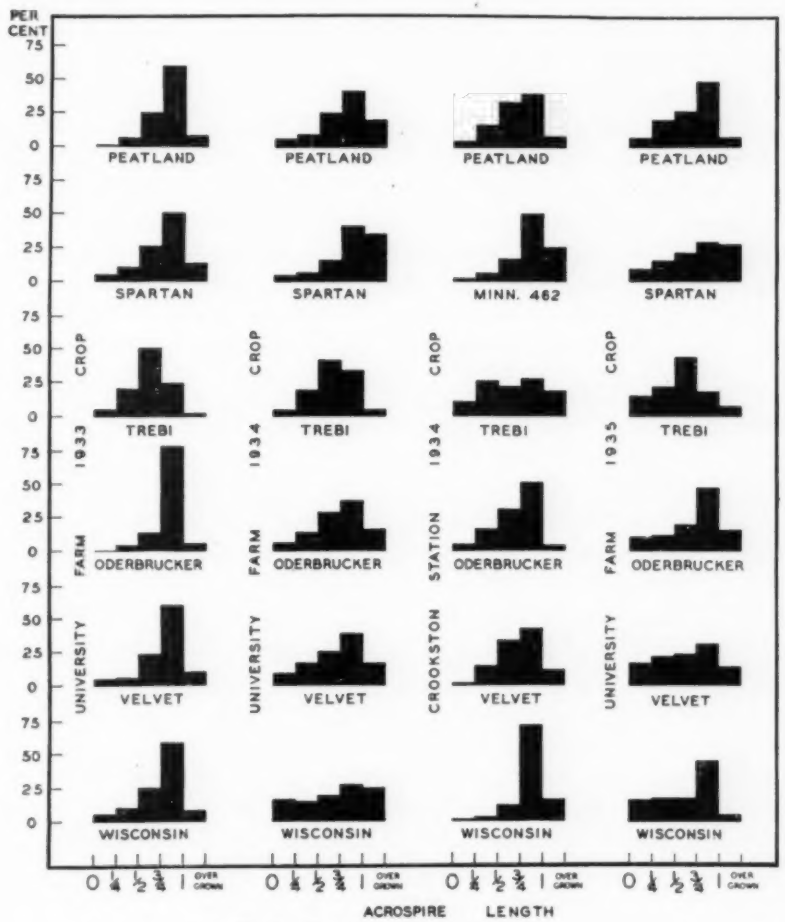

Fig. 5. Growth classification of barley.

Since the different varieties were being germinated under identical conditions in this study it was impossible to allow for the inherent variations in growth capacity due to varietal differences. Therefore, Oderbrucker, which is a variety of proven malting value, was selected as the

control. When, after making a longitudinal section of 100 kernels, it was found that the greatest percentage of acrospires had reached a length between three-quarters and the full length of the kernel, germination was stopped and 100 kernels of the other samples were sectioned.

In making the growth classification the usual procedure of recognizing five groups was followed. Acrospire length was judged by inspection in relation to the kernel length and recorded as being 0 to ¼, ¼ to ½, ½ to ¾, ¾ to 1, and over-grown, depending on the growth. The growth classification for the entire group of samples is recorded graphically in Figure 5, the frequency polygons of which give a general idea of the degree of variability of acrospire growth.

Referring to Figure 5, it is evident that Oderbrucker had the best growth classification in 1933, and Wisconsin No. 38 was best at Crookston. Trebi in all cases had a poor distribution as did all samples grown at University Farm during 1934 and 1935. Wisconsin No. 38 was particularly poor in the 1934 group. This may indicate that this variety is not well adapted to extremely dry growing conditions.

Biochemical Studies

Numerous studies have been made of the development or retardation of enzyme activity during steeping, germination, and drying of barley in malt production. Nordh and Ohlsson (1932) studied the changes in the diastatic properties of barley during malting, noting a slight change in sugar-forming power during the first two days of steeping. More recently Lüers and Rümmler (1933) noted the analogous course of reactions catalyzed by both the liquefying and saccharifying enzymes during malting, which suggests a close connection between the two enzymes. Similar conclusions based on the same type of evidence were earlier presented by Sabalitschka and Weidlick (1929). Lüers (1936) recently presented a very interesting discussion of the enzymatic changes during malting. Although not all of those interested have agreed that two separate enzymes operate in the course of starch hydrolysis to produce reducing sugars and the transformation of starch into products of lower molecular weight, recent work more and more tends to substantiate this view. Ohlsson (1922), Sherman, Caldwell, and Doebbeling (1934), and Doebbeling (1934) have supported this hypothesis.

Notwithstanding the extensive research that has been done on the enzymes involved in malting, a review of the literature reveals no systematic study of varietal differences in the diastatic activity of barley, nor any effort to follow progressively the enzymic changes occurring as barley varieties pass through the green malt and dry malt stages. It was,

therefore, decided to follow the diastatic activity of the barley varieties through the three stages: (*a*) original barley, (*b*) green malt, and (*c*) dry malt. Since no attempt was made to determine the actual concentration of the enzymes present, but only the activity under fixed conditions, it appeared highly desirable to determine also the relative resistance to the action of known quantities of diastase of the starch of the different varieties as malting progressed. The following procedures were used in this study:

Preparation of the samples.—Barley and malt were ground on a Seck mill set at 25, which allows a 0.5 mm. clearance. However, the green malt samples contained nearly 50% moisture and therefore their preparation for analysis presented a problem. The method finally adopted was to place a portion of the green malt in sealed bottles immediately after completion of the germination period and store at a temperature of — 4° C., until analysis was made. Preliminary tests indicated that the freezing effectively stopped further enzymic action without changing appreciably the diastatic activity when the samples were thawed out.

The frozen green malt was prepared for extraction by use of a meat chopper operated in a cold room so that there would be no thawing of the sample until actual extraction had started. Moisture content was determined so that corrections for dry weight of material and proper allowance for the volume of water present in the sample could be made.

Lintner values.—For many years Lintner's (1886, 1887) method for the determination of the diastatic activity of malt has been standard. Lintner values were determined as outlined in the article, "Standard Methods of Analysis" (J. Inst. Brew., **39**: 9 (1933)). In principle, the method involves the determination of the reducing sugars produced under controlled conditions, when a water extract of malt acts on specially prepared potato starch, for 1 hour at 21° C. Two per cent Lintner's soluble starch solution buffered to pH 4.6 was used in this work, and the volumetric method using methylene blue as an internal indicator as proposed by Lane and Eynon (1923) in the Soxhlet (1881) modification of Fehling's (1849) method was adopted.

Diastatic activity (Blish-Sandstedt Method).—Blish and Sandstedt's (1933) adaptation of the Hagedorn and Jensen (1932) method for the determination of maltose produced by autolytic digestion of the sample as proposed by Rumsey (1922) was used to determine the diastatic activity. In this procedure the reducing sugars formed after 1 hour's diastasis at 30° C. in a buffered solution at pH 4.6 were determined in an aliquot of the original solution by the Hagedorn and Jensen (1932) method. The latter is based on the reduction of ferricyanide to ferrocyanide by the reducing sugars.

Wohlgemuth method.—Of the two generally accepted methods for the determination of the starch-liquefying or amyloclastic activity, the method of Wohlgemuth (1908) suitably modified seemed best adapted for this study, although the merit of certain other techniques such as those of Rona and van Eweyk (1924), Jozsa and Gore (1930), Fletcher and Westwood (1930), Chrzaszcz (1931), and Jozsa and Johnston (1935) are recognized. Chrzaszcz and Janicki (1932) made a comparative study of the starch-liquefying powers of malts from various cereals by fifteen different methods of which only six of the methods gave results concordant to within 25%. These results are not surprising in view of the report of Chesley (1931) on the comparison of viscosimetric and Wohlgemuth methods for the estimation of amylase. When equal quantities of enzyme were employed, the calculated values for activity in the presence of different starches ranged from 100 to 2,400% according to Wohlgemuth's method and from 350 to 2,100% according to the viscosimetric method.

The modification of Wohlgemuth's method for the determination of liquefying power of diastase was to add graduated quantities of enzyme solution to 1 cc. of 2% starch solution buffered to pH 4.6, contained in chilled test tubes. The enzyme solution was prepared as in Lintner's determination, by extracting 5 g. of meal with 100 cc. of water for 3 hours at 21° C., stirring every half-hour, allowing the material to settle, and pipetting off the supernatant liquid. In the case of the green and dry malt extracts, it was necessary to dilute 1 cc. of the extract prepared as above to 100 cc., and graduated quantities of this solution were used because in the more concentrated solution it was impossible to measure accurately the small volumes required. The total volume of enzyme solution plus water was kept constant at 5 cc.; therefore, as the volume of enzyme solution varied the difference was made up by adding ice-cold distilled water to the tubes.

After addition of the extract, the tubes were shaken and placed in a 40° C. water bath for 24 hours for the barley samples, and 1 hour for malt samples. The tubes were then placed in an ice bath in order to stop the enzyme action at once, and 10 cc. of ice-cold water plus 1 drop of 0.1 N iodine solution were added. The color standard sheet D, RV normal tone, contained in Mulliken's (1911) color charts was used to indicate the end point which was a clear red color. The color change from blue to red is associated with the degradation of the starch molecule.

Starch resistance to diastatic action.—Diastatic activity may be due to two variables: first, the concentration and activity of the enzymes or their kinases and, second, the resistance of the starch to hydrolysis. In

this study the starch resistance was measured by a modification of the method of Malloch (1929). This method involves inactivation of the natural diastase, the washing out of the excess inactivator, and the determination of the reducing sugars formed under the same conditions as in the Blish-Sandstedt (1933) method when known quantities of taka-diastase were added. The details of the procedure are as follows:

Weigh 5 g. of ground malt or barley (moisture-free basis) into a centrifuge tube of 90 cc. capacity and suspend the meal in about 50 cc. of distilled water. Inactivate the diastase by the addition of 12 to 14 drops of 1:1 sulphuric acid plus 2 cc. of 12% sodium tungstate. Thoroughly stir the contents of the tube, and fill to capacity with distilled water. Centrifuge at 2300 r.p.m. and decant off the supernatant liquid. Add distilled water until the tube is two-thirds full and stir up the mass in the bottom of the tube. Fill the tube to capacity with distilled water and again centrifuge. Repeat washings four times for dry malt and three times for green malt.

After pouring off the last wash water add 32 cc. of buffer solution (3 cc. of glacial acetic acid and 4.1 g. of anhydrous sodium acetate made up to 1 liter with water) at pH 4.7, and 5 cc. of buffer solution containing 0.01 g. of taka-diastase per cubic centimeter, and stir up the contents thoroughly. The reducing sugars formed, after a 1-hour digestion period at 30° C., are determined by the Hagedorn-Jensen (1932) method. In the case of green malt only 30 cc. of buffer solution is added. The average of a large number of determinations showed that, after the washing process was finished, and as much of the wash water removed as possible without loss of sample, there remained in the tube 9 g. of water when the sample was ground barley or dry malt, and 11 g. when green malt was used.

Blank determinations are made in the same manner up to the last washing when 39 cc. (37 cc. in the case of green malt) of 0.4% sulphuric acid and 2 cc. of 12% sodium tungstate are added and the sugar determined as above. The 1-hour digestion period is omitted in the blank.

Since maltose is used to dilute diastatic preparations, it is necessary to run blanks on the taka-diastase solution. An average of several determinations indicated that for a 1-hour digestion period at 30° C., 78 mg. of maltose was present in the solution containing 0.05 g. of taka-diastase. The enzyme solution was freshly prepared shortly before use each time.

Discussion of Results

Table III shows the diastatic activity in degrees Lintner of barley, green malt, and dry malt of the 26 samples. It is difficult to arrive at any definite conclusions regarding varietal differences in the diastatic activity of barleys as expressed by the Lintner test. Considering the four groups in which the samples have been grown in different years and stations, in general, the first three varieties, namely, Wisconsin, Velvet, and Oderbrucker, in each case gave higher Lintner values than the succeeding varieties. The barleys from the Crookston station are slightly higher in diastatic activity than those from University Farm.

The effect of germination is to increase the activity on the average about 54 Lintner degrees. In drying the malt enzymatic activity was reduced in this case below that of green malt by about 19° Lintner.

There is a large variation in results, for in several cases drying produced a slight increase in diastatic activity even though the final drying temperature was 85° C.

TABLE III

DIASTATIC ACTIVITY OF BARLEY AND MALT IN DEGREES LINTNER

Laboratory number	Variety	Year grown	Location	Barley	Green malt	Dry malt
				°L.	°L.	°L.
100	Wisconsin No. 38	1933	University Farm	63	121	119
101	Velvet	"	"	75	116	106
102	Oderbrucker	"	"	77	117	117
103	Trebi	"	"	75	136	137
104	Spartan	"	"	47	91	93
105	Peatland	"	"	46	131	139
106	Wisconsin No. 38	1934	"	72	137	126
107	Velvet	"	"	79	147	157
108	Oderbrucker	"	"	75	157	149
109	Trebi	"	"	86	149	168
110	Spartan	"	"	63	121	107
111	Peatland	"	"	55	144	147
112	Wisconsin No. 38	"	Crookston	84	182	131
113	Velvet	"	"	100	186	120
114	Oderbrucker	"	"	107	238	165
115	Trebi	"	"	96	195	146
116	Minnesota No. 462	"	"	58	133	99
117	Peatland	"	"	87	254	180
118	Wisconsin No. 38	1935	University Farm	63	91	98
119	Velvet	"	"	70	114	108
120	Oderbrucker	"	"	75	155	126
121	Trebi	"	"	64	142	110
122	Spartan	"	"	51	72	87
123	Peatland	"	"	45	145	111
124	Glabron	"	"	58	106	97
125	Minnesota No. 462	"	"	50	88	89

The Lintner tests reported in Table III indicate that it is possible to predict the diastatic activity of the malt from a knowledge of the activity of the barley. The correlation between degrees Lintner of the barley and that of the finished malt was found to be $r = 0.627$. Fisher's (1930) Tables of 5% and 1% points for a value of $n = 26$ are 0.389 and 0.497. Such a large correlation would arise from uncorrelated material, by chance, once in about 1500 times. There is a rather high correlation between the Lintner values of barley and green malt ($r = 0.751$); and between green malt and dry malt the correlation coefficient was $r = 0.799$.

In Table IV is recorded the estimation of the diastatic activity of barley, green malt, and dry malt as determined by the Blish-Sandstedt method. Only two varieties show definite trends by this method. The variety Velvet is consistently high and Peatland is consistently low in diastatic activity as thus determined.

TABLE IV

DIASTATIC ACTIVITY OF BARLEY AND MALT (BLISH-SANDSTEDT METHOD)

Laboratory number	Variety	Year	Location	Maltose per 10 g. of meal per 1-hour digestion at 30° C.								
				Barley			Green malt			Dry malt		
				Total	Blank	Difference	Total	Blank	Difference	Total	Blank	Difference
				Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
100	Wisconsin	1933	Univ. Farm	212	42	170	850	298	552	1590	430	1160
101	Velvet	"	"	230	48	182	995	323	672	1675	465	1210
102	Oderbrucker	"	"	222	48	174	1110	433	677	1730	575	1155
103	Trebi	"	"	198	46	152	805	260	545	1355	365	990
104	Spartan	"	"	202	48	154	790	235	555	1450	330	1120
105	Peatland	"	"	182	46	136	940	300	640	1660	430	1230
106	Wisconsin	1934	"	206	52	154	745	237	508	1440	298	1142
107	Velvet	"	"	202	48	154	665	236	429	1530	323	1207
108	Oderbrucker	"	"	197	48	149	870	325	545	1660	433	1227
109	Trebi	"	"	183	56	127	830	269	561	1385	328	1057
110	Spartan	"	"	200	46	154	785	271	514	1390	293	1097
111	Peatland	"	"	168	46	122	625	229	396	1510	325	1185
112	Wisconsin	"	Crookston	180	46	134	835	335	500	1550	418	1132
113	Velvet	"	"	235	52	183	1005	375	630	1620	405	1215
114	Oderbrucker	"	"	219	56	163	915	345	570	1555	428	1127
115	Trebi	"	"	201	48	153	920	270	650	1215	288	927
116	Minnesota No. 462	"	"	210	48	162	845	242	603	1575	478	1097
117	Peatland	"	"	192	72	120	965	320	645	1510	408	1102
118	Wisconsin	1935	Univ. Farm	192	41	151	483	153	330	1470	348	1122
119	Velvet	"	"	280	40	240	535	145	390	1600	557	1043
120	Oderbrucker	"	"	264	44	220	844	293	551	1640	648	992
121	Trebi	"	"	193	40	153	665	200	465	1355	408	947
122	Spartan	"	"	278	48	230	518	180	338	1245	325	920
123	Peatland	"	"	225	48	177	786	213	573	1520	542	978
124	Glabron	"	"	235	45	190	620	180	440	1436	520	916
125	Minnesota No. 462	"	"	224	40	184	628	165	463	1410	483	927

In contrast to the Lintner values, the diastatic activity of barley determined by the Blish-Sandstedt method cannot be used to predict the activity of the malt made from the barley. The correlation between the diastatic activity of barley and malt in this case gave a value of $r = -0.403$. There is no correlation between the values for barley and green malt nor between green malt and dry malt.

Two methods have been used in this study to determine diastatic activity of barley, green malt, and dry malt, but unfortunately the results of the two methods do not agree. For example, the correlation between the diastatic activity of barley as determined by Lintner and Blish-Sandstedt methods is $r = -0.145$, and the same comparison for the malt is $r = 0.338$. Neither figure is significant. There really is very little reason for the supposition that the two values are related, for while both measure "diastatic activity," in a sense, the methods used in the measurement are entirely different. In one case (Lintner) the enzymatic preparation from malt acts on soluble potato starch and in the other (Blish-Sandstedt) on the raw starches native to the malt. Because of the fact that in brewing malt enzymes are used to hydrolyze starches from a large number of cooked cereals, it has generally been thought, in the past, that Lintner's method gave an indication of the malt's potential converting power. Recently this method has come into rather general disrepute because of the peculiar and abnormal substrate used. The Blish-Sandstedt method, however, especially for studies of barley varieties, has many merits such as speed, accuracy, and the fact that the value indicates the action of the barley or malt enzymes on their own substrate.

The reducing sugars in the different barley varieties is rather constant as the blank determinations indicate. The maltose reported in the blank is determined in the sample before the period of active enzymatic digestion. During germination the maltose content increases about six times as evidenced by the much larger blank determination. The drying process also caused the formation of some maltose. Except for the barley sample Peatland No. 117 there is not much variation in the barley blanks. In green and dry malt Oderbrucker gives consistently high results and Spartan low. No other varietal differences are discernible.

Drying produced a tremendous increase in diastatic activity as measured by maltose productions during the digestion period. This is rather surprising when it is remembered that the malt was maintained at a temperature of 85° C. for 10 hours. Since no attempt has been made in this investigation to determine the concentration of diastase present, it is difficult to interpret the above findings. Evidence here presented (Table V) seems to indicate that perhaps the resistance of the starch to en-

zymatic action may be the cause for the differences between the green and dry malt and not necessarily an increase in enzyme. Assuming no change in enzyme activity due to drying, on the basis of the starch resistance there should be an increase in maltose produced from the dry sample. There is a significant correlation between the Blish-Sandstedt values of green malt and the resistance of the green malt starch to diastatic action ($r = 0.523$), and also between the diastatic activity of malt and the starch resistance ($r = 0.609$).

There may have been, however, a change in the concentration of enzymes during heating. If the contention of Waldschmidt-Leitz and Putt (1931), that enzymatic processes are controlled and vary in intensity not so much by formation and destruction of enzyme as by the influence of specific activators, is correct, then perhaps heating favors activation. These authors believe that proteolytic action is responsible for the production of the activator.

The resistance of starch to diastatic action is a problem of primary importance. Alsberg (1926) and Malloch (1929) have pointed out that differences in starch resistance are usually attributed to the influence of environmental conditions during the time the starch was formed or to variations in the mechanical process used to separate the starch. The second of these factors is not important here because no attempt was made to separate the starch in pure form. Even though three crop years and two locations are represented in the barley samples studied there does not appear to be any noticeable varietal influences. The 1935 samples are as a crop very much more resistant than the other crops as is evident from an inspection of Table V.

It will be noticed in Table V, that a constant blank value has been subtracted from the total maltose column for all values. The justification for this is that after the process of washing out the inactivating agent the maltose that remains unremoved is practically a constant. This constant will vary with the washing procedure, but since the washing technique was standardized the justification for subtracting the mean of a number of determinations become evident.

The large increase in starch resistance of green malt as compared to the barley and dried malt was hardly expected. It is probable, in view of the work of Alsberg and Griffing (1925), on the influence of fineness of grinding on diastatic activity, there is no justification for comparing the green malt starch resistance values with the other two sets of data. It was impractical to grind the green malt samples, containing nearly 50% moisture, to the same degree of fineness as the dry samples. The tendency, however, must be for the malting process to decrease the susceptibility of starch to diastatic action, because the barley and malt

TABLE V

RESISTANCE OF BARLEY AND MALT STARCH TO DIASTATIC ACTION AS MEASURED BY THE MALTOSE PRODUCED WHEN 0.05 GRAM OF TAKA-DIASTASE ACTS ON 5 GRAMS OF MEAL

Laboratory number	Sample	Maltose per 10 g. of meal per 1-hour digestion at 30° C.								
		Barley			Green malt			Dry malt		
		Total	Blank	Difference	Total	Blank	Difference	Total	Blank	Difference
		Mg.	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
100	Wisconsin	256	83	173	151	101	50	239	81	158
101	Velvet	232	83	149	153	101	52	201	81	120
102	Oderbrucker	233	83	150	170	101	69	219	81	138
103	Trebi	245	83	162	158	101	57	247	81	166
104	Spartan	272	83	189	157	101	56	235	81	154
105	Peatland	237	83	154	162	101	61	193	81	112
106	Wisconsin	242	83	159	143	101	42	225	81	144
107	Velvet	209	83	126	149	101	48	219	81	139
108	Oderbrucker	240	83	157	160	101	59	245	81	164
109	Trebi	226	83	143	150	101	49	209	81	128
110	Spartan	236	83	153	140	101	39	216	81	135
111	Peatland	220	83	137	159	101	58	197	81	116
112	Wisconsin	252	83	169	195	101	94	270	81	189
113	Velvet	252	83	169	159	101	58	261	81	180
114	Oderbrucker	246	83	163	193	101	92	229	81	148
115	Trebi	282	83	199	162	101	61	245	81	164
116	Minnesota No. 462	270	83	187	217	101	116	197	81	116
117	Peatland	226	83	143	178	101	77	172	81	91
118	Wisconsin	179	83	96	126	101	25	118	81	37
119	Velvet	170	83	87	135	101	34	107	81	26
120	Oderbrucker	183	83	100	138	101	38	111	81	30
121	Trebi	193	83	110	102	101	1	102	81	21
122	Spartan	194	83	111	112	101	11	104	81	23
123	Peatland	178	83	95	107	101	6	96	81	15
124	Glabron	187	83	104	103	101	2	103	81	22
125	Minnesota No. 462	178	83	95	112	101	11	120	81	39

samples were ground on the same mill, and yet the dry malt has a greater starch resistance. It may be that the drying process dehydrated the ground particles to the extent that during the washing and subsequent digestion period the malt preparation does not have sufficient time to reach the same stage of particle accessibility as that of the barley meal.

The amyloclastic, or liquefying, action of barley and malt extract was studied by use of a modification of the Wholgemuth (1908) method.

TABLE VI

AMYLOCLASTIC ACTIVITY OF BARLEY AND MALT BY WOHLGEMUTH METHOD

Laboratory number	Variety	Year	Location	Number of cubic centimeters of 2% starch solution hydrolyzed by 1 cc. of barley extract		
				Digestion periods		
				24 hours at 40° C.	1 hour at 40° C.	
				Barley	Green malt	Dry malt
100	Wisconsin No. 38	1933	Univ. Farm	0.77	66.6	55.6
101	Velvet	"	"	1.67	68.9	66.7
102	Oderbrucker	"	"	2.00	100.0	83.3
103	Trebi	"	"	0.35	58.8	58.7
104	Spartan	"	"	0.74	52.6	47.6
105	Peatland	"	"	1.11	95.3	83.2
106	Wisconsin No. 38	1934	"	0.74	45.4	40.0
107	Velvet	"	"	0.69	57.1	76.8
108	Oderbrucker	"	"	0.77	60.7	47.7
109	Trebi	"	"	0.32	142.8	83.4
110	Spartan	"	"	0.42	55.6	35.7
111	Peatland	"	"	0.53	66.7	76.9
112	Wisconsin No. 38	"	Crookston	0.55	62.5	34.5
113	Velvet	"	"	0.71	83.4	47.6
114	Oderbrucker	"	"	0.62	86.9	60.6
115	Trebi	"	"	0.31	52.7	33.4
116	Minnesota No. 462	"	"	0.42	50.0	47.6
117	Peatland	"	"	0.71	100.0	86.9
118	Wisconsin No. 38	1935	Univ. Farm	0.98	27.8	43.4
119	Velvet	"	"	3.33	40.0	55.7
120	Oderbrucker	"	"	10.00	62.5	58.7
121	Trebi	"	"	0.59	32.3	37.1
122	Spartan	"	"	1.67	35.8	25.0
123	Peatland	"	"	12.50	28.6	58.8
124	Glabron	"	"	3.33	33.3	43.5
125	Minnesota No. 462	"	"	2.50	25.1	34.5

The results of the application of this method to barley, green malt, and dry malt is recorded in Table VI. It is evident that after a 24-hour period at 40° C. sufficient starch has been liquefied by the enzymes

extracted from barley to be significant although as noted by Chrzaszcz and Janicki (1923) the liquefying action of resting seeds of cereals is almost entirely lacking. The striking result of this study is the very low starch-liquefying power of the enzyme preparation from Trebi barley. In every case this variety gave the lowest value but this characteristic is changed during the germination period. The correlation coefficient between the amyloclastic activity of barley and green malt is only $r = -0.027$, and between barley and dry malt, $r = 0.040$. There is a very significant correlation between the amyloclastic activity of green malt and dry malt ($r = 0.720$).

There is no significant correlation between the Lintner value of barley and the amyloclastic activity of barley as $r = -0.284$. The same type of comparison between Lintner values of green malt and Lintner values of dry malt yields the following correlation coefficients: $r = 0.474$ and $r = 0.631$. This latter value is significant.

It should be noticed that the data recorded for barley and that for green and dry malt in Table VI are not on the same basis. The liquefying powers of the green malt and dry malt extract, if expressed on the same basis as the barley liquefying units, are from about 500 to 1000 times as great.

Summary

Samples of the same barley varieties were obtained from the increase plots at University Farm, St. Paul, for the year 1933, 1934, and 1935. Because of the severe drouth conditions at University Farm in 1934, additional samples were obtained from the Crookston, Minnesota substation. The ash, protein, 1000 kernel weight, weight per bushel, and per cent germination of all the barley samples are compared.

A detailed study was made of the rate of imbibition of water by these barley varieties. Also the growth of acrospire classification during germination for the same barley samples was studied.

The saccharifying action of the aqueous extract from barley, green malt, and dry malt was studied by both the Lintner and Blish-Sandstedt methods. The liquefying action of the same extracts as used in the Lintner procedure was determined by a modification of Wohlgemuth's method. The relative resistance of the starches of the barley varieties was determined at the green malt and dry malt stage, as well as in the barley; and, in addition, the reducing sugars formed during the malting process were followed.

Conclusions

For a normal steeping period of about 50 hours the variations in imbibition rates for different barley varieties were small. The variety Spartan absorbed water at a slower rate than the six-row barleys. The

varieties Oderbrucker and Peatland imbibed water at a more rapid rate than the other varieties studied. The variety Trebi absorbed water at an average rate for the first few hours, but later this rate was reduced.

The barleys grown at Crookston, Minnesota, imbibed less water per unit time than did the same varieties grown at University Farm.

Of the varieties tested, Oderbrucker gave the most uniform acrospire growth during germination. Trebi barley had the slowest rate of acrospire growth. The acrospire growth classification of Wisconsin No. 38 for the exceedingly dry year of 1934 was very poor.

The diastatic activity of barley and malt is correlated when measured by the Lintner method, but not when the Blish-Sandstedt method is employed. Neither is there any correlation between the results of the two methods when applied to either malt or barley samples.

Two barley varieties showed definite trends in diastatic activity as measured by the Blish-Sandstedt method. The variety Velvet was consistently high and Peatland consistently low. By use of Lintner's method no varietal differences in the diastatic activity of barleys was noticeable.

Malting and drying increased the resistance of starch to the action of taka-diastase. There was a significant negative correlation between the diastatic activity of barley, as measured by the Blish-Sandstedt method, and starch resistance for barley; but a positive correlation existed between the Blish-Sandstedt values for green and dry malt and starch resistance. No varietal differences in starch resistance of barleys to enzymatic action were found.

Trebi barley was decidedly deficient in liquefying power, but when germinated it became normal in this respect. There were no other varietal differences. The liquefying power of malt was not changed appreciably by drying while the saccharifying power increased by this process. The liquefying action of barley could not be correlated with the saccharifying action of the same barleys as determined by the Lintner method.

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FURTHER STUDIES ON THE ACTIVITY OF PROTEINASE IN FLOUR[1]

A. K. Balls and W. S. Hale

Food Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

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The existence of a protein digesting enzyme in flour, noted by Balland (1884), has been demonstrated many times through hydrolysis of the flour protein itself (autolysis), as measured by changes in soluble amino nitrogen, total amino nitrogen (Swanson and Tague, 1916), and viscosity (Ford and Guthrie, 1908). Furthermore, the liberation of tryptophane from egg albumin (Baker and Hulton, 1908) and the liquefaction of gelatin (Stockham, 1920) have been observed following the admixture of flour. Finally, Cairns and Bailey (1928) extracted flour with water, precipitated the extract with safranine, and thus obtained a ferment preparation that digested casein.

Experiments at the U. S. Bureau of Chemistry and Soils have shown that the autolysis of flour proteins is increased by the addition of cysteine or glutathione, thus indicating that the protein-splitting enzyme is of the papain type (Balls and Hale, 1935). The enzyme is largely resident in the bran and germ. Similar activation of the hydrolysis of casein by the water-soluble enzyme confirmed this conclusion (Balls and Hale, 1936). The activity of oxidizing agents in improving the appearance of bread, the effect of oxidants used in bleaching flour, and the improvement that flour undergoes on storage in air can be adequately explained as resulting from the oxidation of the cysteine-like activator. A diminution in the proteolytic activity of the dough is a consequence of such treatment, with the result that the dough of the treated flour does not become as soft as the dough of the untreated and proteolytically more active flour. The difficulties found in preparing a light, spongy loaf of bread from whole wheat flour, or from flour containing wheat-germ are probably in part due to the fact that such materials are much richer in proteinase (or else in activator) than is normal white flour.

Independently of us and at about the same time, Jörgensen (1935) reached conclusions similar to ours as the result of able research on

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the effect of oxidants in the dough. While our work has been more concerned with the effect of bleaching agents on flour, the principle involved appears to be the same. We take pleasure in acknowledging Dr. Jörgensen's work, and in pointing out the mutual corroboration thus afforded.

The chemical changes in flour that can be attributed to the action of the native proteinase appear to be very small as measured by any of our present day methods. On the other hand, changes to be observed in the physical condition of the dough are very striking. Among them is an extraordinary increase in fluidity. This increase can be very simply demonstrated by a procedure that might perhaps furnish the basis of a good technical method.

The rate at which a heavy object such as a shot falls through a viscous medium has been used by Ladenburg (1907) to measure the viscosity of the medium. It is easily observed that the time required for a small lead shot to sink below the surface of a paste of flour and water varies greatly with the character and previous treatment of the flour.

Changes in the fluidity of these flour pastes are far more rapid and striking than the hydrolysis of the amino nitrogen which we have previously reported. For example, a paste of whole wheat flour after 30 minutes incubation (30° C.) presented a surface through which a B. B. shot took 6 minutes to sink. After the same flour had been "bleached" with chlorine, the shot required 5 hours. The addition of traces of cysteine (1 mg. cysteine-HCl per g. of dry flour) accelerated the liquefaction of pastes from both the untreated and the chlorine-treated samples. The time then taken for the submergence of the shot was 20 seconds for the untreated and 80 seconds for the chlorine-treated flour. The submergence time was reduced from 6 minutes to 20 seconds in the case of the unbleached flour, and from 5 hours to 80 seconds in the case of the bleached flour (see Table I).

TABLE I

METHOD OF DETERMINING RATE OF SOFTENING OF FLOUR PASTE

Whole Wheat Flour; 5 g. flour + 5 cc. CO_2 – saturated water; temp. 30° C.
B. B. shot dropped on surface of paste from a height of 5 inches.

Age of paste when shot was dropped	Time required for immersion of shot			
	Unbleached flour	Bleached flour	Unbleached flour + 1 mg. cysteine per g. flour	Bleached flour + 1 mg. cysteine per g. flour
Time	*Time*	*Time*	*Time*	*Time*
30 min.	6 min.	5 hrs.	20 sec.	80 sec.

This change in fluidity is what might be expected from the action of a proteinase that could be inactivated by chlorine and reactivated by cysteine. However, it is not a foregone conclusion that this liquefaction of the paste is due to a proteolytic ferment. That the proteins, rather than other constituents of the paste, undergo change follows from the fact that gluten behaves in a similar way.

If one undertakes to prepare gluten by the usual method from flour to which cysteine has first been added, the result is less gluten and softer gluten than is normally extracted. If gluten prepared in the ordinary way is subsequently treated with cysteine, it becomes soft and sticky after a short incubation. On kneading this gluten again with tap water, an appreciable amount is lost, but the residue becomes much stiffer, though never quite so rubbery as the original gluten. A second treatment with cysteine again produces the softening effect. A second washing removes more protein. The process may be repeated until all the gluten is gone.

In order to study the effect of cysteine on gluten more closely, the conditions of this experiment were changed, with the intention of exaggerating the results. This may be done by kneading comparatively large amounts of solid cysteine hydrochloride into the gluten, and then incubating at 45° C. for 30 to 60 minutes.

If enough cysteine is used the result is a complete liquefaction of the gluten to a milky white fluid that can be diluted indefinitely with water. Under these conditions 40 g. of wet gluten made from unbleached white flour was found to liquefy in less than 30 minutes when treated with 100 mg. cysteine hydrochloride. Dilution with ten volumes of water, however, produced coagulation of part of the protein. A similar experiment, with 200 mg. cysteine-HCl to 40 g. of gluten, produced a liquid that only threw down a small precipitate, largely starch granules, when diluted with water to any desired extent (see Table II).

The so-called " peptization of gluten " that occurs on treatment with salts such as potassium iodide has been frequently studied. While such salts disperse no inconsiderable quantities of protein, their effect appears to differ from that of cysteine. With comparable amounts of potassium iodide, for instance, the extent of disintegration does not even approach that produced by cysteine. Furthermore, some of the properties of the cysteine-dispersed gluten lead us to believe that changes have taken place in chemical constitution as well as in the state of aggregation.

The greater part of the gluten so suspended in water has evidently not suffered a severe breakdown. Electrolytes such as NaCl, Na_2SO_4, HCl, and the like, even when added in very small quantities, cause coagulation of the protein. The coagulum is a sticky mass resembling freshly precipitated gliadin, without, however, being completely soluble in 70%

TABLE II

DISPERSION AND ELECTROLYSIS OF GLUTEN

100 g. gluten (= 5.000 g. N.) was dispersed with 1 g. cysteine-HCl (= .089 g. N.), final dilution being 300 cc. The supernatant liquid after centrifuging (3250 r.p.m.; diam. head 25 cm.; 3 min.) contained 4.842 g. nitrogen, showing that 4.85% of the nitrogen was still neither dissolved nor dispersed. The supernatant liquid was then diluted to 535 cc. with sodium chloride solution; the resulting system thereafter contained 1.0 g. NaCl per 100 cc. The precipitate was collected, redissolved in 500 cc. water and precipitated again by adding salt solution sufficient to give a concentration of roughly 1% NaCl. The second salt precipitate was washed with 50 cc. H_2O merely to remove excess salt, and then dissolved in 375 cc. The solution contained 3.317 g. N. or 65.18% of the N. originally used as gluten; 250 cc. of this solution was used in the electrolysis described below.

Electrolysis
2000–3500 V.; 10–1.0 m. amp.; total time 72 hrs.; total current approx. 360 m. amp. hrs.

	N. at start	N. at end	Test for SH at end	Test for S–S at end
Beaker No. 1 (anode)		0.001	−	−
2	0.00	0.008	−	−
3	0.00	0.069	−	+
4	1.1095	0.485	+	+
5	1.1095	1.478[1]	−	+
6	0.00	0.225[1]	−	+
7	0.00	0.009	−	−
8 (cathode)	0.00	0.001	−	−
	Total 2.219 g.	2.275 g.		

[1] Solid separated here—Total N. on solid and liquid.

alcohol. The amount of the coagulum increases with increasing salt concentrations, until in 10% sodium chloride only about 3% of the original nitrogen remains soluble. The coagulum from a freshly prepared suspension of gluten "dissolves" in warm water, however, and may be repeatedly precipitated and "redissolved." After three such precipitations the coagulum was dried and powdered but was still found to be soluble.

TABLE III

CYSTEINE DISPERSED GLUTEN[1] + NaCl

Volume of dispersed gluten (cc.)	Amount of NaCl (g. per 100 cc.) (total volume)	Nitrogen on supernatant liquid (mg.)
5.00	0.1	15.80
5.00	0.5	6.17
5.00	1.0	4.77
5.00	5.0	3.91
5.00	10.0	2.94
5.00	None	38.52

[1] 100 g. wet gluten + 500 mgs. cysteine-HCl finally diluted to 500 ml. 5 cc. of the diluted gluten (containing 38.52 mg. N.) was mixed with a solution of NaCl in the proper proportion to give a volume of 20 cc. at the salt concentration stated. The precipitate was centrifuged off and N determinations (Kjeldahl) made on the supernatant liquid.

The dispersed gluten may also be boiled without coagulating the protein. Heat coagulation does not even occur after oxidation of the -SH group with an excess of hydrogen peroxide, but if the oxidized solution is precipitated with salt and the protein then dried and powdered, it is no longer completely soluble in water. After several precipitations with salt, the coagulum still gives a strong positive test for sulphydryl groups with nitroprusside.

Long continued electrolysis with a high voltage current (in an apparatus similar to that first used by Williams (1935), did not succeed in removing the substance responsible for the nitroprusside reaction. This furnishes some evidence that the -SH content of the protein is not wholly a matter of adsorbed cysteine.

Arguments for a chemical change in the gluten as a result of treatment with cysteine may be summarized as follows:

1. The coagulum produced by salt bears no resemblance to the original gluten in physical properties (plasticity and solubility in water) nor in chemical properties (coagulation by heat).

2. The nitrogen not precipitated by sodium chloride seems to us higher than might be expected from gluten, even if this could be finely divided in water.

3. Removal of -SH groups from the treated protein was not accomplished by repeated dispersion in water and precipitation with salt, nor by long continued electrolysis.

4. The rapid development of a peculiar mercaptan-like odor in the mixture of cysteine and gluten is evidence that some sort of a chemical reaction takes place.

It has been mentioned that many of the ordinary reagents used to " peptize " gluten do not produce effects comparable to those of cysteine. Similar though not identical effects do result, however, from the treatment of gluten with certain other substances. Of the reagents we have tried, glutathione, potassium cyanide, sodium sulphite, sodium sulphide, and sodium hydroxide have in small amounts produced extensive disintegration of the protein. Gluten is undoubtedly hydrolyzed by alkali, however, so the effect of sodium sulphide and sodium hydroxide can be disregarded here. On the other hand, slightly acidified solutions of both sulphite and cyanide are effective. These substances disintegrate the gluten rapidly at first, and apparently better at room temperature. In a few minutes, however, a second reaction appears to occur which results in a coagulation. This is not due simply to the electrolyte present because the coagulum is not soluble after washing with water. The change has the appearance of a polymerization, for the gluten remaining insoluble assumes a peculiar rubbery state. Nevertheless, part of the protein remains in " solution " and may be coagulated by addi-

tional salt or acids. Some observations were therefore made on these materials in the hope that a clue to the action of cysteine might be found.

Cyanides and sulphites, like cysteine and glutathione, are strong reducing agents. They are well-known activators of proteinases of the papain type. This activation results from the reduction of disulphide linkages in the enzyme to sulphydryl groups. It was found that acidified cyanide or sulphite quickly dissolved from the gluten some substance giving a positive nitroprusside reaction for -SH. This substance appears no longer to be a protein. The gluten after incubation with either cyanide or sulphite was diluted with water, and the insoluble coagulum centrifuged out. The supernatant liquid gave positive tests for -SH after precipitation of more protein by NaCl, by HCl (to pH 3.5) or both together. Precipitation with tungstic acid, however, removed all of the sulphhydryl compound in the case of cyanide treatment, but left it in solution after the sulphite treatment. The -SH carrying substance is not cysteine, however, since it gave negative tests by the method of Sullivan (1926). Details are shown in Table IV, where it is apparent that several other well-known "peptizing" agents do not split off either S-S or -SH in soluble form from gluten. One may conclude from the experiments with cyanide and sulphite that here the gluten has actually undergone a chemical change. This change is a protein breakdown, evidenced by the separation of an -SH-containing body more soluble than the original protein. The -SH body is still fairly complex, but apparently breaks down further in the presence of sulphite than of cyanide.

Discussion

It is evident that a special type of protein breakdown occurs in gluten under the influence of the strong reducing agents cyanide and sulphite, and by analogy may occur with cysteine and glutathione. Quantitative measurements are under way that may decide the latter point.

We are apparently dealing with a reduction of the gluten proteins, for the amount of reductant required is far greater than that needed to activate a small quantity of proteinase. Proteins in which the S-S linkages have been reduced are no novelty. Mirsky and Anson (1935) both reduced and oxidized the sulphur linkages in several proteins, and described methods for determining -SH and S-S groups. More recently Linderström-Lang and Duspiva (1935) have shown that the clothes moth is able to digest insoluble keratin, because the protein is first reduced to the -SH form by a glutathione-like substance in the intestine. The -SH form is readily attacked by the ordinary digestive

TABLE IV

EFFECT OF SEVERAL PEPTIZING AGENTS ON GLUTEN

20 g. gluten was kneaded with the reagent in 5 cc. water; stood for 30 min. at 45° C., then diluted to total volume of 50 cc. with H_2O

Reagent	Nitroprusside test on supernatant liquid	S-S, shown by nitroprusside test after reduction with KCN	Control containing same solution + 0.05 mgs. cysteine	Nitroprusside test on supernatant liquid after addition of NaCl to 10% centrifugation, acidification to pH 3.5–4 and recentrifugation	Nitroprusside test on tungstic acid filtrate		Sullivan test on tungstic acid filtrate
					Nitroprusside	S-S[2]	
HCl 50 mg.	0	0	+				
KI 100 mg.	0	0	+				
NaCNS 100 mg.	0	0	+				
Urea 500 mg.	0	+	+				
NaOH 100 mg.	+		+	+			
NaCl 100 mg.	0	0	+				
Trypsin 20 mg.	0	+	+				
$CaCl_2$ 100 mg.	0	+	+				
Reagents neutralized to approximately pH = 6.0							
Na_2SO_3 200 mg.	+		+	+[3]	+	+	±
KCN 100 mg.	+		+	+[3]	0	0	0
Trypsin 20 mg. + KCN 100 mg.	+		+	+[3]			
Urea 500 mg.	0	+[1]	+				

[1] Extract contained soluble protein.
[2] Stronger test after KCN, indicates cysteine as well.
[3] Gluten partly peptized and coagulated again.

enzymes. Also Michaelis (1935) has shown that the solubility of keratin in thioglycollic acid and similar reducing agents is due to a reduction of S-S to -SH groups. The -SH protein (keratein from keratin, by anology with cysteine from cystine) has been separated out and studied. It is easily digested.

On the other hand, the scission of a sulphur-containing group from a protein has also been observed. Jones and Gersdorff (1934) have shown that casein treated even with diluted alkali loses cystine. In our experiments with gluten, it appears as though we have a combination of both processes, the reduction of S-S to -SH, and the breaking off of a sulphur-containing fragment from the rest of the protein complex. It is not apparent whether the sulphur is detached as S-S, or first reduced to -SH.

Are we to consider the dispersion of the gluten and sulphur-containing protein fragment as catalysed by the flour proteinase?

Against the enzymic explanation is the fact that the breakdown does not continue further on incubation, for samples of peptized gluten show no increase in titratable amino (or carboxyl) groups after incubation for 2 days more. If an enzyme takes part in the decomposition it is specific for this step or it has been completely inhibited. The former case seems unusual for a proteinase, the latter predicates the production of a powerful inhibitory substance from the flour. We have not been able to demonstrate the existence of such an inhibitor. If it were present, the addition of flour to a system containing a papainase and a digestible protein should show at least some inhibition of the breakdown of the digestible protein. This is not the case. Neither flour nor cysteine-treated gluten proved able to inhibit the digestion of casein by papain. Furthermore, both flour and peptized gluten were themselves digested rapidly by added papain, and the sum of the NH_2 groups produced by the enzyme on each substrate alone exactly equalled the amount of digestion found when the enzyme acted in a mixture of the two. There is evidently no inhibitory substance present—at least none of a well-recognized type. It must be admitted on the other side, that heated gluten does not react with cysteine as we have described, but this may be due to a rearrangement of the protein structure. Further, an actual splitting of the protein takes place accompanied by a visible change in physical properties. The addition of a proteinase to the system of course intensifies this behavior, but that might also be expected in any case. If enzymic at all, the effect on gluten may be comparable to that of rennin on milk. In reality, our present criteria of proteinase action are fulfilled, but it is also apparent that these criteria are here insufficient.

It is also evident that the large quantities of reducing agent necessary for complete dispersion of the gluten point to an effect on the protein itself rather than on a small amount of admixed enzyme. Thus, while the flour proteinase present is doubtless activated by the cysteine, sulphite, and other reducing substances in these experiments, it is most unlikely that the whole effect of these reducing substances is limited to the enzyme. The researches cited on the digestion of keratin, however, clearly indicate that the reduction of the protein may render it more easily attacked by protein-digesting enzymes, so that even if the effect of cysteine is directly on the protein, indirectly it is an important factor in the enzymic splitting. Most probably there are two distinct processes to consider, the action of the cysteine on the protein and on the enzyme. While the two processes run simultaneously, it is evident that a small amount of cysteine would affect the enzyme, and a larger amount the gluten proteins as well.

We should like therefore to present to you the effect of cysteine on gluten as a phenomenon, not of the activation of an enzyme, but of the "activation of a protein."

Acknowledgment

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ON THE PRESENCE OF GLUTATHIONE IN WHEAT GERM

B. Sullivan, Marjorie Howe, and F. D. Schmalz

The Russell-Miller Milling Company Laboratory, Minneapolis, Minnesota

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In a previous paper from this laboratory (Sullivan *et al.*, 1936) it was shown that the deleterious effect of wheat germ on the baking quality of flour was due to some compound present in the water extract of the germ. Several compounds known to be present in germ were tried and it was found that none of these was responsible for the injurious action which wheat germ manifests.

Grassmann and his co-workers (1931) have shown that plant, as well as animal, intracellular proteinases are activated by organic sulfhydryl compounds such as cysteine or reduced glutathione. Hellerman and Perkins (1934) think it possible that the activation of papain may be due to a reduction of the S–S groups in the enzyme molecule to S–H form. Jørgensen (1935, 1936) and Balls and Hale (1935) have shown that although the proteinases of flour are normally more or less latent, they show very marked activity when activated by cysteine or glutathione. Both these authors believe that the effect of potassium bromate, Agene, and other oxidizing agents is due to the inactivation of the proteolytic enzymes of the flour. Balls and Hale (1936) have also confirmed the conclusion of others that the proteinase, like other enzymes in wheat, is present in greater amounts in germ and outer layers of the grain than in the endosperm.

Experimental

In continuing our work on the deleterious effect of germ extract, it was found that the water extract of germ gave a very positive nitroprusside reaction. The Sullivan test was negative for free cysteine but was positive for glutathione (Sullivan and Hess, 1931). Glutathione was isolated from fresh, especially purified wheat germ by a modification of the method of Regnier (1933). 0.26 g. of impure glutathione was obtained from 1 kg. of germ (dry basis). By the iodine titration method of Weller (1935), 0.4605% of glutathione was found in freshly prepared wheat germ. In attempting to purify the substance, it was found very difficult to remove all inorganic impurities, which caused a

small percentage of ash to remain after combustion. Owing to the inorganic impurity which contaminated the product (4 to 9% in various preparations), the figures for nitrogen (theoretical 13.68%, found 12.53%), for hydrogen (theoretical 5.57%, found 5.20%), and for carbon (theoretical 39.06%, found 32.46%) were too low. There is no doubt, however, that the substance in question is glutathione since the material (60 mg.) had the same bad effect on the farinograph curve of our standard flour and in the baking test as the original water extract of germ. The impure material shows a very strong nitroprusside reaction.

The farinogram of a standard patent flour is shown in Figure 1 as Curve 1. Curve 2 illustrates the action of the same flour when the water extract of 30 g. of germ is added to the dough. Curve 3 shows the action of the standard flour when 60 mg. of glutathione obtained from wheat germ after recrystallization of the compound from absolute alcohol and drying in a vacuo several days was added to the dough. Curve 4 shows the reaction of the standard flour when 60 mg. of glutathione similarly prepared from fresh bakers' yeast was added to the dough. Table I records the baking data.

TABLE I

EFFECT OF VARIOUS GLUTATHIONE PREPARATIONS ON THE BAKING QUALITY OF A STANDARD PATENT FLOUR

Sample	Treatment	Dough quality	Volume, 100% basis
Standard Patent	None	Strong, elastic	100
Standard Patent	Water extract from 10% fresh germ	Very poor, very soft	75
Standard Patent	60 mg. glutathione from germ	Poor, soft, sticky	79
Standard Patent	60 mg. glutathione from yeast	Poor, soft, sticky	75
Standard Patent	10% fresh germ	Poor, soft, dead	72
Standard Patent	10% fresh germ plus .06 g. potassium bromate	Fair plus	89

It may be of some interest to record the results of the nitroprusside test on various milling separations:

Short Patent	doubtful reaction
Clear	doubtful reaction
Low Grade	faint reaction
Bran	faint reaction
Germ	very positive reaction

The water extract of germ plus potassium bromate gave a faint test. On standing 10 minutes the reaction was negative.

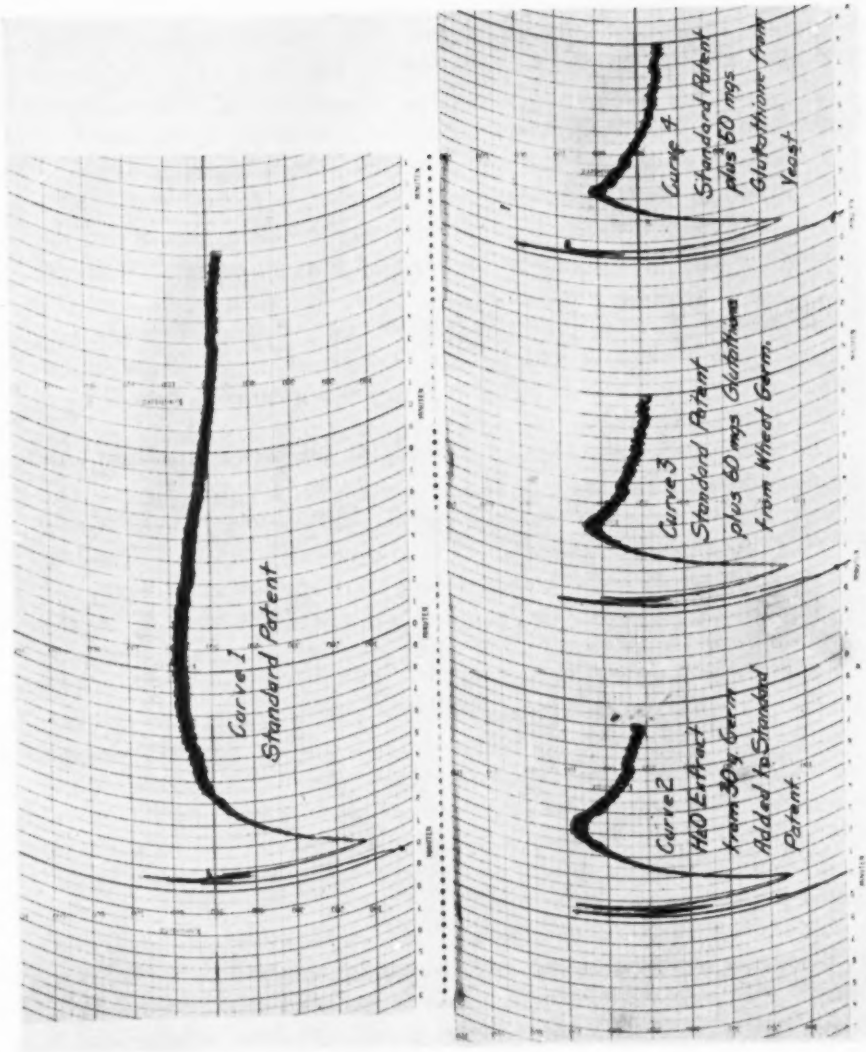
Curve 1
Standard Patent
Curve 2
H2O Extract from 30% Germ Added to Standard Patent
Curve 3
Standard Patent plus 60 mgs Glutathione from Wheat Germ
Curve 4
Standard Patent plus 60 mgs Glutathione from Yeast

Figure 1.

It is now shown that this activator, reduced glutathione, is concentrated in the germ, and that patent flour contains only traces, if any, of this compound. The fact that it is glutathione in germ, rather than the proteolytic enzyme itself, which is the cause of the injurious action of the germ, can be proven by boiling the water extract for 10 to 15 minutes. This should inactivate the proteolytic enzymes of germ. However, the heated extract still has an injurious effect on dough quality. This is because the heated extract of germ still contains glutathione, which, in turn, activates the latent proteinases of the flour. It has also been shown that the water extract on standing loses its effect, the rate of loss being dependent on the temperature and the time. This behavior is characteristic of glutathione, the probability being that the reduced form of glutathione is oxidized on standing at room temperature in water solution.

Numerous investigators have proved that potassium bromate and Agene minimize the injurious action of the germ. Some additional results are given in Table I. The oxidizing agents simply change some of the S–H glutathione to the S–S form.

In spite of all the evidence recently accumulated on the subject of the activation of the proteolytic enzymes of flour by substances containing the sulfhydryl group, the authors believe that too narrow a view of this explanation of changes in flour quality should be avoided. As a matter of fact, the colloidal state of the proteins of flour can be appreciably modified by many substances. Any change in the oxidation-reduction potential of flour, whether by SH activation of proteinases or by other means, will modify the gluten considerably. The $-S-H- \rightleftarrows -S-S-$ system, although seemingly quite specific for the proteinases of the papain type, is not the only system which can cause marked changes in the physical properties of the flour proteins. Many inorganic substances, sodium sulfite for instance, by lowering the oxidation potential of the flour-water system, can exert as bad an effect as glutathione. Potassium bromate can reverse this effect. Other oxidation-reduction systems, both organic and inorganic, can modify the colloidal behavior of the gluten proteins. It is rather interesting to observe in this connection that cysteine, which contains the specific S–H group, causes a very marked deleterious action as measured by the farinograph. However, unlike glutathione, cysteine permits of the production of a normal loaf of bread.

The measurement of the oxidation-reduction potential of flours and the effect on their oxidation-reduction systems of such oxidizing substances as bromate, Agene, peroxides of the fatty acids, and of reducing substances as well as certain reversible systems, is now being studied,

and will be reported in the near future. We are also endeavoring to prepare in a pure ash-free state the glutathione isolated from wheat germ.

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REPORT OF THE MALT ANALYSIS STANDARDIZATION COMMITTEE

D. A. COLEMAN, *Chairman*

United States Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, June 1936)

This year's committee consisted of D. A. Coleman, Chairman, J. A. Anderson, H. C. Gore, L. E. Leatherock, Elsie Singruen, H. W. Rohde, and J. D. Veron.

The recommendation of last year's committee, that the methods for the analysis of barley malt sponsored by the American Society of Brewing Chemists [1] be studied for the purpose of determining their suitability for adoption as standard methods of analysis by this Association, was carried out. Supplementing this activity, efforts were

[1] Official Methods of the American Society of Brewing Chemists. Published by A.S.B.C., Chicago (1935).

TABLE I

RESULTS OF PHYSICAL AND CHEMICAL DETERMINATIONS MADE ON A SAMPLE OF MALT AS ANALYZED BY THE METHODS OF THE AMERICAN SOCIETY OF BREWERY CHEMISTS

By 17 Collaborators

Collaborator	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	21	22	Average
Physical Examination																		
Bushel weight, lbs.	40.0			40.9	39.0	39.0	39.0	39.0	39.5	39.5	39.5							39.5
Length of acrospire																		
0–¼	3			4	3	3	4	4	1	0	3							2.7
¼–½	3			2	0	2	1	1	2	0	5							1.8
½–¾	2			0	1	1	3	1	4	12	17							4.5
¾–1	90			94	93	88	88	85	89	86	51							85.0
Overgrown	2			0	3	6	4	9	4	2	24							6.0
Foreign seeds, %	0.1			—	1.82	1.25	1.0	—	.50	—	.4							0.7
Broken kernels, %	0.1			.8		.7	.6	—	.25	—	1.0							0.25
Mold, %	none			1.0	none	none	none	none	none	none	none							none
Weight per 1000 kernels, grams																		
As is	33.2			32.4	32.0	33.2	33.6	33.7	33.4	32.8	33.6							33.1
Dry basis	35.4			30.4	30.0	31.2	31.5	31.7	31.4	30.8	31.5							31.9
Mealiness, %																		
Mealy	88			99	96	98	89	87	95	88	90							92.0
Half mealy	10			0	3	2	9	9	4	10	7							6.0
Glassy	2			0	1	0	2	4	1	2	3							2.0
Assortment, %																		
on 8/64 inch sieve	54.5			—	—	40.7	27.3	—	28.2	—	—							37.7
on 7/64 inch sieve	24.5			73.2	72.1	33.3	50.0	—	44.1	—	—							49.5
on 6½/64 inch sieve	16.2			22.0	23.2	20.4	18.9	—	21.3	—	—							20.3
on 5½/64 inch sieve	3.6			3.9	3.5	3.8	3.1	—	5.1	—	—							3.8
on 5/64 inch sieve	.5			.5	.5	1.2	.3	—	.9	—	—							.65
through 5/64 inch sieve	.7			.4	.7	.6	.4	—	.4	—	—							.53
Chemical Analysis																		
Moisture, %	6.0	6.2	6.2	6.2	6.2	6.1	6.1	5.9	5.	6.	[illegible]	6.1	6.2	6.2	6.0	6.1	6.2	6.1
Extract—fine grist, %																		
As is	70.1	70.3	70.1	70.1	69.1	69.2	70.1	69.7	69.8	69.9	69.5	71.2	69.6	70.8	69.8	69.8	69.9	69.9
Dry basis	74.6	74.9	74.7	74.8	73.7	73.6	74.6	74.1	74.2	74.3	74.1	75.9	74.2	75.4	74.3	74.3	74.6	74.5
Extract—coarse grist, %																		
As is	68.7	69.4	—	—	67.8	—	—	—	—	—	68.8	—	—	—	—	—	—	68.6
Dry basis	73.1	73.9	—	—	72.3	—	—	—	—	—	73.4	—	—	—	—	—	—	73.2
Difference—fine over coarse, %	1.5	1.0	—	—	1.4	—	—	—	—	—	.7	—	—	—	—	—	—	1.1
Conversion in minutes	5.0	—	—	5	5	5	5	5	5	5	5	—	5	—	—	—	7	5.0
Color of wort	1.6	—	—	1.75	—	1.6	1.75	1.7	1.5	1.75	49 [6]	—	1.4	—	—	—	1.5	1.6
Odor of mash	A [1]	—	—	A	SA [2]	SA	SA	A	A	SA	SA	—	A	—	—	—	A	A
Degree of clarity	C [3]	—	—	C	C	B [7]	C	C	C	C	B	—	C	—	—	—	C	C
Speed of filtration	N [4]	—	—	N	N	N	N	N	N	N	N	—	N	—	—	—	N	N
Diastatic power (Lintner value)																		
As is	—	—	—	—	111	—	111	—	—	—	—	—	—	—	—	—	—	111
Dry basis	126	108	102.7	124 [5]	118	115	118	121	118	130.5	143 [8]	138	126	158	113	134	—	124.5

[1] Aromatic. [2] Slightly aromatic. [3] Clear. [4] Normal. [5] No buffer. [6] Stamner Units. [7] Brilliant. [8] Polarimetric determination.

made by the Committee to determine the extent of variability in the methods of analysis used for the evaluation of malt adjuncts.

To accomplish the activities just mentioned, a sample of malt of average physical and chemical characteristics was sent to 17 collaborators with the request that they analyze the malt by the methods of the A.S.B.C.

At the same time, a sample of brewers' corn flakes, and a sample of brewers' rice was sent to 16 and 13 collaborators, respectively, with the request that these malt adjuncts be analyzed by the different collaborators in the manner usually practiced by the collaborator.

Collaborative Studies with Barley Malt

The results obtained by the collaboration on the sample of malt are recorded in Table I. From an examination of these data it would appear that there is sufficient concordance between the results obtained by the majority of the 17 collaborators, with the exception of the data for the Lintner value determination, to justify the adoption of the methods of the A. S. B. C. as official, first reading, by this Association. This is particularly true of the chemical analyses.

With respect to the physical examination of the malts, the only determination which appeared to be troublesome was that of test weight per bushel. With proper testing equipment, however, this variable should quickly disappear.

Collaborative Studies with Brewers' Corn Flakes

The collaborators' findings for tests made on brewers corn flakes are listed in Table II.

Although the 16 collaborators used 5 different methods for the determination of the moisture content as well as the fat content of brewers' corn flakes, and 3 different methods for the determination of extract, the resulting analytical figures were surprisingly uniform. This can be seen from an examination of the data listed in Table II.

For determining the moisture content of corn flakes, most laboratories reported the method sponsored by the Association of Official Agricultural Chemists,[2] which method is an exact replicate of that sponsored by the A.S.B.C. for the determination of the moisture content of barley malt.

The methods for the analysis of the fat content of the corn flakes varied largely with respect to solvent used and time of extraction. Preparation of the sample, however, was practically uniform.

Inasmuch as most of the methods used in this study by the collaborators, for the analysis of corn flakes, were a derivation of those

[2] Journal of the Association of Official Agricultural Chemists, Vol. 19, No. 1 (1936).

TABLE II

CHEMICAL ANALYSIS OF BREWERS' CORN FLAKES
By 16 Collaborators

Collaborator	2	3	5	7	8	9	10	11	14	15	16	17	18	19	20	21	Average
Moisture, %	8.7	8.6	8.1	8.2	8.4	8.5	8.4	8.6	8.1	8.2	8.7	8.5	8.3	8.0	8.3	8.4	8.4
Fat, %	.36	.30	.37	.38	.42	.38	.54	.38	.44	.36	.32	.50	.45	.35	.58	.53	.43
Extract, %																	
As is	84.8	85.3	84.7	85.1	85.3	86.0	85.3	85.6	84.4	86.1	[1]	83.2	[1]	[1]	[1]	84.5	85.0
Dry basis	92.9	93.3	92.2	92.7	93.1	94.0	93.0	93.7	92.0	93.8	95.0	91.0	[1]	[1]	[1]	92.3	93.0
Method used																	
Moisture	[2]	[2]	[3]	[4]	[4]	[4]	[4]	[4]	[5]	[4]	[6]	[7]	[7]	[4]	[4]	[6]	
Fat	[8]	[9]	[3]	[10]	[11]	[11]	[12]	[11]	[12]	[3]	[13]	—	[12]	[10]	[10]	[14]	
Extract	[15]	[15]	[3]	[16]	[16]	[16]	[15]	[16]	[16]	[14]	[14]	[14]	[1]	[1]	[1]	[14]	

[1] Not determined.
[2] 5 hours in vacuum oven at 100° C.
[3] Not disclosed.
[4] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 90.
[5] 1 hour in vacuum oven at 100° C.
[6] Wahl-Henius method.
[7] 1 hour at 130° C. in air-oven.
[8] 6 hours with ethyl ether, moisture-free sample.
[9] 6 hours with carbon tetrachloride, moisture-free sample.
[10] Method of Association of Official Agricultural Chemists (moisture-free sample extracted with ethyl ether for 6 hours, dried and weighed) see Jour. A.O.A.C. (1936) **19**: 91.
[11] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 91.
[12] Method of Association of Official Agricultural Chemists (moisture-free sample extracted with ethyl ether for 16 hours, dried and weighed) see Jour. A.O.A.C. (1936) **19**: 91.
[13] 10-gram sample extracted with carbon tetrachloride for 20 hours.
[14] Wahl-Henius method, see Wahl-Henius Handy Book of Malting and Brewing (1908 ed.), p. 554.
[15] Own method—varies from A.O.A.C. method in respect to preparation of sample.
[16] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 91.

sponsored by the A.O.A.C., it is believed that the methods of the A.O.A.C. for the analysis of corn flakes should be used by this Association as tentative methods, with the objective of later adopting the methods, as future collaborative study warrants, as the official methods of this Association.

Collaborative Studies with Brewers' Rice

The results of the analysis of the sample of brewers' rice which was sent to 13 collaborators are recorded in Table III.

TABLE III

CHEMICAL ANALYSIS OF BREWERS' RICE
By 13 Collaborators

Collaborator	2	3	5	7	8	9	10	11	14	17	18	20	21	Average
Moisture, %	13.3	13.2	12.9	12.1	12.5	12.3	12.6	13.0	12.9	13.4	12.2	12.2	11.9	12.7
Fat, %	.56	.53	.55	.63	.48	.61	.51	.34	.53	.57	.63	.40	.55	.53
Extract, %														
As is	85.9	86.2	81.8	79.1	84.2	83.6	83.1	84.1	81.7	84.5	1	1	84.4	83.5
Dry basis	99.0	99.3	93.8	90.0	96.2	95.3	95.0	96.6	93.8	97.6	1	1	95.7	96.9
Method used														
Moisture	2	2	3	4	4	4	4	4	5	6	6	4	15	
Fat	7	8	9	10	10	9	11	10	10	9	11	10	15	
Extract	12	12	13	14	14	14	13	14	14	15	1	1	14	

[1] Not determined.
[2] 5 hours in vacuum oven at 100° C.
[3] Not disclosed.
[4] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 91.
[5] 1 hour in vacuum oven at 100° C.
[6] 1 hour in 130° C. air-oven.
[7] 6 hours with ethyl ether; moisture-free sample.
[8] 6 hours with carbon tetrachloride; moisture-free sample.
[9] Not disclosed.
[10] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 91.
[11] 16 hours extraction with ethyl ether; moisture-free sample.
[12] Own method; varies largely with respect to treatment of rice before inversion by malt.
[13] Not disclosed.
[14] Method of Association of Official Agricultural Chemists, see Jour. A.O.A.C. (1936) **19**: 91–92.
[15] Method of Wahl-Henius Institute.

The widest variation in analytical data of the three collaborative samples was experienced with the brewers' rice. This was due, in part, to the character of the lot of rice used, as the material contained an appreciable quantity of oil-bearing weed seeds. Analytical procedure, however, was heavily responsible for some of the wide variances.

There was too wide a variation in the amount of moisture found by the collaborators in the sample; the range being 2.4%. Four different methods were reported as being used to determine moisture.

The analyses for fat were quite ragged, due somewhat to the foreign material present in the sample. Methods also contributed to the analytical situation. Here, again, several different procedures varying with respect to solvent and preparation of sample were used to determine the fat content of the sample.

The widest variation in the analytical data occurs in the determination of extract. A range of 9% was experienced.

In view of the fact that there was considerable variation in the manner in which the rice starch was gelatinized before being acted upon by the malt diastase, it is believed that this point in the determination is a potential source of error.

With but one exception, all those collaborators who used the methods of analysis for brewers' rice sponsored by the A.O.A.C. reported analytical data which were fairly concordant. For this reason, therefore, it is recommended that the methods for the analysis of brewers' rice, sponsored by the A.O.A.C., be used by this Association as tentative methods for the analysis of brewers'rice with the objective of later adoption if future collaborative study warrants, as the official methods of this Association.

Recommendations

It is recommended:

That the methods of analysis of the American Society of Brewering Chemists for barley malt be adopted by this Association as official (first reading), with the exception of the method for the determination of diastatic power (Lintner value).

That the method for the determination of diastatic power is to be held in a "tentative" status, and is to be given an additional year's study before recommendations with respect to its adoption by the Association are agreed upon.

That the methods for the analysis of malt adjuncts now being sponsored by the Association of Official Agricultural Chemists as "tentative," be adopted by this Association as tentative methods for the purpose of further study.

THE EFFECT OF CHEMICAL FLOUR IMPROVERS ON PROTEOLYTIC ACTION IN RELATION TO THE GAS-RETAINING CAPACITY OF FERMENTING DOUGHS

J. T. FLOHIL

Pillsbury Flour Mills Company, Minneapolis, Minnesota

(Read at the Annual Meeting, June 1936)

Introduction

It is now generally understood that a great deal of the baking quality of flour depends on the gas-producing and gas-retaining capacities of the fermenting dough. The gas-producing capacity depends mainly on the availability of fermentable sugar during the process of fermentation. The gas-retaining capacity is determined by the quantity and physical condition of the gluten, although perhaps the starch complex of the dough may influence gas retention to some extent.

Until very recently there has been considerable speculation as to what determines the physical condition of gluten and why certain chemicals have in many cases such a pronounced improving effect on the baking quality of flour.

At first these chemical improvers were believed to stimulate the activity of the yeast, resulting in an increased gas production, which in turn should explain the improvement in the baking quality. That this view was held about 10 years ago is evidenced by Bailey's "Chemistry of Wheat Flour," page 232.

We know now that this theory is erroneous, as subsequent investigations have shown that there is really no difference in the gas production of doughs made with treated and untreated flours, providing of course that these flour improvers do not also contain ammonium compounds, which are yeast foods. On the other hand, it has been conclusively proved that flour improvers of the non-yeast food type, generally speaking, positively affect the gas-retaining capacity of the dough, when such improvers are applied in suitable quantities.

The explanation of the action of these improvers on the dough has led to considerable speculation and has involved certain colloidal phenomena as well as other factors not directly related to flour protein, *i.e.*, the fat-like substances, the so-called lipids, to which reference will be made later.

To Balls and Hale (1936) of the U. S. Department of Agriculture, and Holger Jørgensen (1935) of Denmark, must go the credit of first having called our attention to the effect of chemical bread improvers on the proteolytic enzymes of flour. These investigators proved this effect to be directly related to the baking quality of flour.

Jørgensen also noted that flour improvers of the oxidizing type—bromates, iodates, and the like—decrease the solubility of the flour proteins, indicating that the proteolytic enzymes of the flour are at least made partly inactive by these agents.

He also found that these same flour improvers inhibited the proteolytic action of papain on gelatine and that these improvers caused a remarkable decrease in the solubility of the protein in papain-treated flour.

Jørgensen also discovered that flour improvers caused a decrease in the proteolytic action of the flour enzymes on gelatine.

Although Jørgensen has proved his theory quite satisfactorily, we have considered it desirable to extend his investigations using a different technique.

Experimental

Effect of Oxidizing Agents on Proteolytic Activity

Experiment I: If it be true that chemical improvers really neutralize the harmful effect of an excess of proteolytic enzymes in flour, it should be possible with the help of such improvers to restore the baking quality of a flour which has been purposely ruined by the addition of a powerful proteolytic enzyme, *e.g.*, papain. A flour of normal baking quality was selected and to it was added .005% papain (Merck). This treatment affected the baking quality very adversely. The original baking quality of the flour was restored with the help of a typical flour improver, potassium bromate.

The results of the baking tests made in Experiment I are shown as Figure 1.

From this experiment it appears that improvers of the bromate type actually inhibit the detrimental effect of an excess of certain proteolytic enzymes in the flour. A similar action is to be noted for the use of nitrogen tri-chloride gas.

In order to study further the effect of proteolytic action on wheat gluten and the inhibitory effect of bromates, other experiments were undertaken.

Experiment II: Equal amounts of washed gluten were divided into small pieces, transferred to test tubes and treated as described in the legend of Figure 2. The sequence of the tubes is left to right.

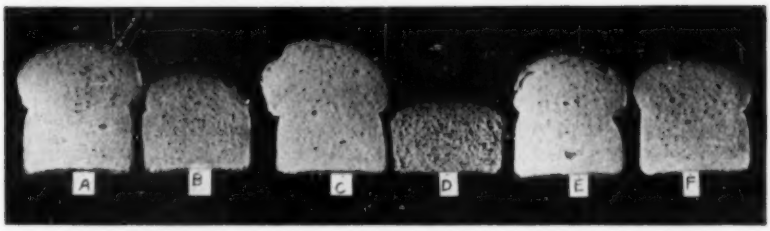

Fig. 1.

Loaf A, untreated flour (control).
Loaf B, same flour as A plus .005% papain.
Loaf C, same combination as B plus .0018% $KBrO_3$.
Loaf D, same flour as A plus .0075% papain.
Loaf E, same combination as D plus .0027% $KBrO_3$.
Loaf F, same combination as B, but bleached with 7 g. NCl_3 per barrel.

To prevent bacterial action 15 mg. of boric acid was added to each tube. The tubes were set away for 48 hours at a temperature of 30° C. The photograph shown as Figure 2 was taken at the end of 48 hours. It is evident that the gluten in the third tube was entirely broken down by the papain, whereas its effect was absolutely neutralized by the presence of bromate as shown by the condition of the second tube. The first tube shows the presence of a certain quantity of proteolytic enzyme which naturally exists in washed gluten.

EXPERIMENT III: In the following experiment, papain was not used; instead, the proteolytic enzymes naturally present in malted wheat flour were utilized. To prepare the malted wheat flour proteases a

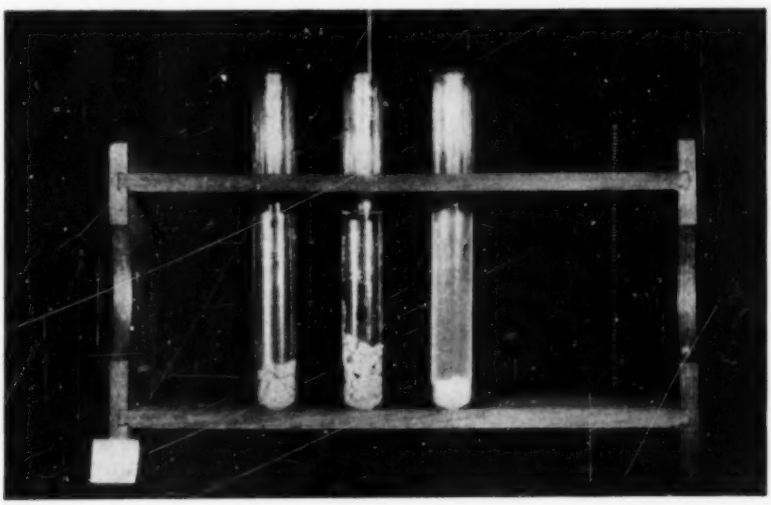

Fig. 2.

Tube 1, distilled water only.
Tube 2, distilled water plus 27 mg. of papain and 15 mg. of $KBrO_3$.
Tube 3, distilled water plus 27 mg. of papain.

20% suspension of malted wheat flour in water was made and later centrifuged in order to obtain a clear solution. This solution was then used in the experiment. Boric acid was added to each tube as in Experiment II to prevent bacterial action.

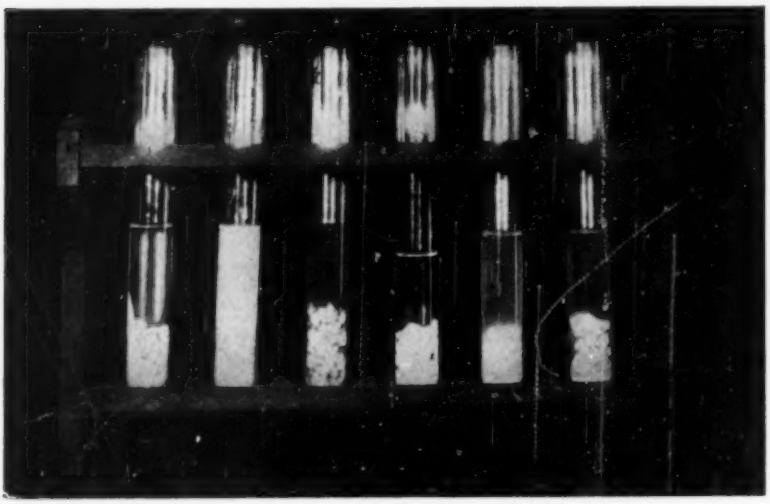

Fig. 3.

Tube 1, washed gluten in water.
Tube 2, washed gluten in malted flour solution.
Tube 3, washed gluten in malted flour solution plus 15 mg. $KBrO_3$.
Tube 4, boiled egg white in water.
Tube 5, boiled egg white in malted flour solution.
Tube 6, boiled egg white in malted flour solution plus 15 mg. $KBrO_3$.

The treatment given each tube is to be found in the legend attached to Figure 3. The sequence of the tubes is left to right.

The effect of the proteolytic enzymes of malt flour on washed gluten is to be noted from tube 2. The same effect is to be observed on egg white (tube 5). The presence of bromate in tubes 3 and 6 prevented proteolytic action almost completely. In this experiment the action of the proteolytic enzymes on boiled egg white has been introduced as this is a rather pure protein. Washed gluten always encloses a considerable amount of starch and the objection could be made that perhaps the diastatic action of the malted flour extract might be responsible for the breaking down of the washed gluten complex and not necessarily the proteolytic enzymes. Such a possibility, however, is excluded in the case of the egg white, which does not contain starch.

EXPERIMENT IV: The test-tube technique for studying proteolytic activity, as here described, suggested a means for studying the behavior of other well-known flour improvers as well as for finding other yet unknown flour improvers. With this idea in mind the following set

of experiments concerning the action of some typically well-known flour improvers as well as that of an apparent improver, not generally known (potassium thiocyanate), was made. The results obtained are shown as Figure 4; the legend recording the treatment given to each tube. The sequence of tubes is left to right.

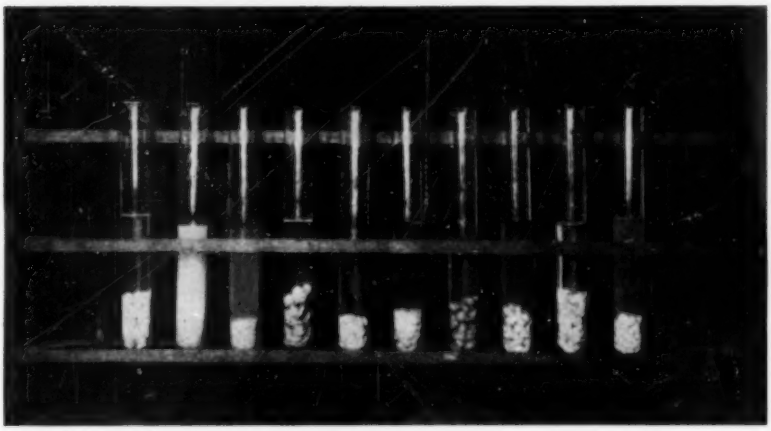

Fig. 4.

Tube 1, gluten in distilled water.
Tube 2, gluten in malted flour extract, about 10 cc.
Tube 3, gluten in malted flour extract, plus 30 mg. $KClO_3$.
Tube 4, gluten in malted flour extract, plus 30 mg. $NaBO_3$.
Tube 5, gluten in malted flour extract, plus NCl_3.
Tube 6, gluten in malted flour extract, plus 30 mg. $(NH_4)_2S_2O_8$.
Tube 7, gluten in malted flour extract, plus 30 mg. KIO_3.
Tube 8, gluten in malted flour extract, plus 30 mg. $KBrO_3$.
Tube 9, gluten in malted flour extract, plus 30 mg. $KMnO_4$.
Tube 10, gluten in malted flour extract, plus 30 mg. KCNS.

It is again evident that if no improver is added to the malted-flour extract, as in tube 2, the gluten completely loses its original character through disintegration.

The $KClO_3$ in tube 3 was undoubtedly an oxidizing salt. Experience, however, has shown that it is not an efficient flour improver. This is confirmed by its action on the gluten as shown in the photograph. The remaining tubes contained well-known flour improvers, with the exception of the last tube which contained KCNS. KCNS also inhibited proteolytic activity.

EXPERIMENT V: Although KCNS is not an oxidizing salt, the following baking tests proved the test tube experiment to be of real value with respect to identifying flour improvers. The action of KCNS as a flour improver is shown in Figure 5. The percentages of KCNS are given in the legend of Figure 5. The sequence of the loaves is left to right.

However, the importance of the improving action of KCNS must be considered entirely academic, as it is a rather poisonous chemical.

EXPERIMENT VI: The following experiment was undertaken to show that certain chemicals, which have elements in common with well-known flour improvers but which have no improving effect themselves,

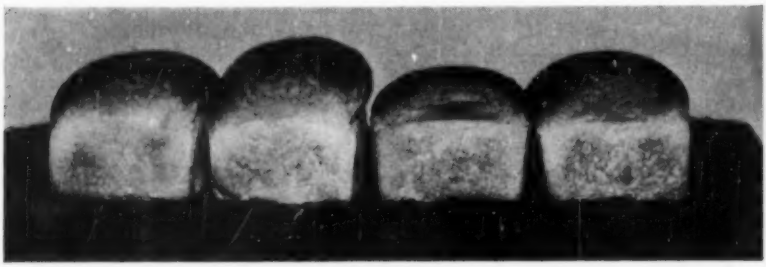

Fig. 5.

Loaf 1, made from an unbleached patent flour.
Loaf 2, made from an unbleached patent flour plus ½% KCNS.
Loaf 3, made from an unbleached clear flour.
Loaf 4, made from an unbleached clear flour plus ½% KCNS.

also do not prevent proteolytic disintegration of either gluten or egg white. The results obtained in this experiment are shown in Figure 6 and the chemicals used given in the legend attached thereto. The sequence of the tubes is from left to right.

It is evident that the appearance of tubes 2 and 8 containing $KBrO_3$, which is a very powerful improver, contrasts sharply with the appear-

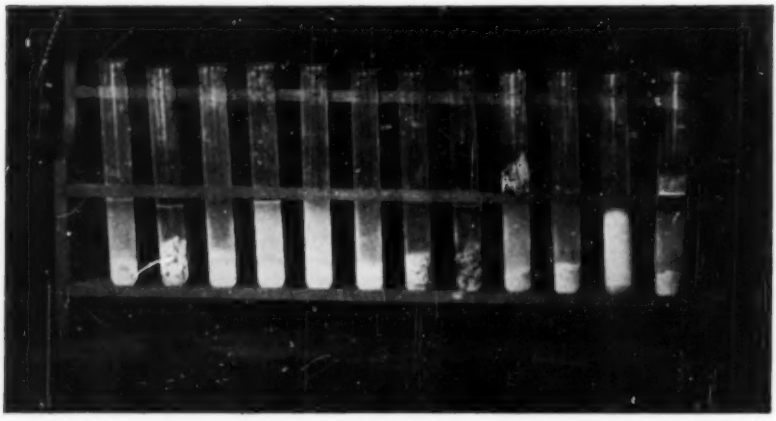

Fig. 6.

Tube 1, malted flour extract plus 30 mg. KCNS.
Tube 2, malted flour extract plus 30 mg. $KBrO_3$.
Tube 3, malted flour extract plus 30 mg. NaCl.
Tube 4, malted flour extract plus 30 mg. $KClO_3$.
Tube 5, malted flour extract only.
Tube 6, malted flour extract plus 30 mg. KBr.

(Egg Whites)

Tube 7, malted flour extract plus 30 mg. KCNS.
Tube 8, malted flour extract plus 30 mg $KBrO_3$.
Tube 9, malted flour extract plus 30 mg. NaCl.
Tube 10, malted flour extract plus 30 mg. $KClO_3$.
Tube 11, malted flour extract only.
Tube 12, malted flour extract plus 30 mg. KBr.

(Washed Glutens)

ance of tubes 6 and 12 which contained KBr. It is known that neither NaCl nor KBr can be considered as bread improvers in the ordinary meaning of the term.

The experiments just described apparently confirm the general conclusions of Jørgensen.

Measurement of Proteolytic Activity in Dough

If the degree of proteolytic action in a dough could be accurately measured, a valuable means of judging the baking quality of the flour would be available. So far the Brabender farinograph, Swanson recording dough mixer, Chopin extensimeter, and similar types of mechanical dough testers are the only means available which graphically register the effect of proteolytic action in dough. Chemical methods have to date been unsuccessful.

Development of a Method

It has been found possible to make a rather useful comparison between the relative proteolytic state of different flours by observing the glutens washed from them.

For this purpose, however, it is necessary to modify the old-fashioned method of gluten washing so as to allow sufficient time for the proteolytic enzymes to act on the gluten (corresponding to the time these enzymes act on the gluten during the fermentation process). For this reason the flours are made into flour-water doughs and set aside for at least 4 hours at a temperature corresponding to that used in the fermentation process, before attempting to wash out the gluten.

EXPERIMENT VII: The usefulness of this method may be illustrated by the following experiment.

Four samples of flour were made up as follows:

No. 1, untreated patent
No. 2, same as 1 plus 4% malted wheat flour
No. 3, same as 2 plus 2 mg. $KBrO_3$ per 100 g. flour
No. 4, same as 3 plus 12 mg. $KBrO_3$ per 100 g. flour.

Two sets of doughs were made from these flour samples. The first set of doughs was used in order to obtain the washed gluten by the old-fashioned washing process, in which the gluten is washed shortly after the doughing procedure. The second set of doughs was set at a temperature of 30° C. for a period of 4 hours, before the gluten was obtained by washing. The washed glutens were then formed into balls and placed on a glass plate and left undisturbed for 1 hour. To prevent the glutens from drying, they were covered with inverted beakers. After 1 hour's rest, they were photographed as shown in Figures 7 and 8 with the following results:

Fig. 7. Glutens washed directly after doughing.

Fig. 8. Glutens washed after the doughs had been left to stand at 30° C. for 4 hours.

Figure 7 represents the glutens washed directly after doughing, while Figure 8 represents the glutens washed after the doughs had been kept at 30° C. for 4 hours. It will be noted that the condition of the glutens in the first procedure does not readily reflect the proteolytic condition of the 4 flours as indicated by their respective treatments. The photograph of the second series clearly demonstrates the softening effect due to proteolysis and the restoring effect of increasing amounts of bromate. These effects are more forcefully demonstrated in Figure 9 where the effects of the inclusion of considerably larger quantities of malted wheat flour are illustrated. Correspondingly more bromate was also used.

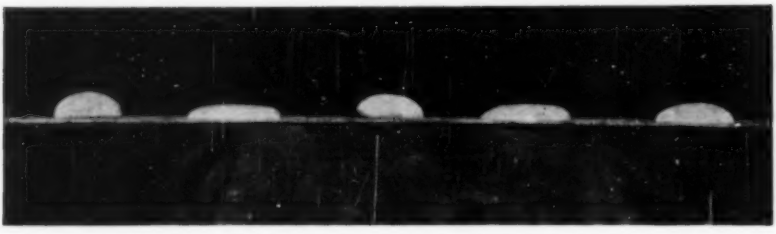

Fig. 9. The flours from which these glutens were washed were treated as follows (reading from right to left):

Gluten 5, an untreated Southwestern patent.
Gluten 4, same as 5 with 6% malted wheat flour added.
Gluten 3, same as 4 treated with .05% bromate.
Gluten 2, same as 5 with 12% malted wheat flour added.
Gluten 1, same as 2 treated with .1% bromate.

Location of Proteinases in the Wheat Kernel

Conceding that an excessive amount of proteolytic enzymes harms the gluten-complex and consequently the baking quality of a flour, it appears to be in order to inquire into the location of these enzymes in the wheat kernel. The germ is naturally thought of first. Logically the most active biological factors of the wheat kernel, the enzymes, are concentrated in the germ.

The wheat germ was found to be exceedingly rich in proteolytic enzymes, as will be seen later on in this article. The fact that the presence of a liberal amount of germ in flour very adversely affects its baking quality has long been known. It is rather surprising that this effect was only recently connected with the proteolytic action of the germ (by Holger Jørgensen, 1935).

Much more attention has been given to the rather high lipid content of wheat germ in connection with this harmful quality, as is evident from numerous references in the literature on this subject. There is proof indeed that comparatively large amounts of lipids can affect the baking quality adversely. It will be shown in the following experiments that the effect of proteolytic enzymes on the baking quality is of such magnitude that the influence of the lipids in wheat germ can be safely subordinated, even ignored.

EXPERIMENT VIII: Pure wheat germ was ground sufficiently fine to pass through a 9XX sieve. This finely ground germ was then extracted first with alcohol and then with ether in order to free it completely from all oil and lipids.

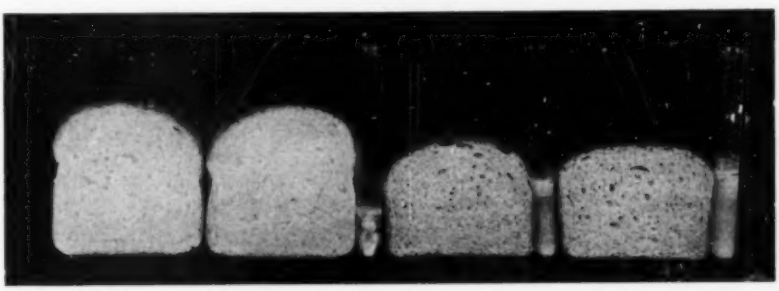

Fig. 10. Loaf 3 was obtained from the standard flour containing 2% of natural wheat germ and loaf 4 contained 2% of the extracted germ. Loaf 1 is the control, and loaf 2 is the loaf obtained from the standard flour to which ether-alcohol extract was added in the same amount as could be extracted from the same amount of wheat germ used in loaf 3.

Two percent of this extracted germ was added to a standard flour and baked in comparison with the same flour to which 2% of the unextracted germ was added. The resulting loaves did not differ materially as regards volume and general appearance, as is evident from the appearance of the loaves shown in Figure 10.

The proteolytic tests shown were made with water infusions of the extracted oil, the unextracted and extracted wheat germ. The proteolytic action of the non-extracted and the extracted germ as demonstrated by these tube tests confirm the baking results.

The extracted oil was incorporated in the flour used in the bake and represented by loaf 2 in the following way:

A calculated amount of oil was dissolved into ether and mixed with enough starch to result in a mixture containing an equal amount of germ oil as occurred in 2% of wheat germ on the basis of the flour used.

The ether was then evaporated and the resulting starch-oil combination was added to the flour. This same combination was used in preparing the infusion used in test tube 1, Figure 10.

EXPERIMENT IX: As judged by the comparative baking tests, neither alcohol nor ether had any effect on the proteolytic potency of the enzymes. This was also found to be true in the instance of papain. It was observed that papain and the proteolytic enzymes in wheat germ are rather resistant to dry heat. For instance, no lessening of the proteolytic effect of wheat germ was found after drying it for 16 hours at a temperature of 100° C. (Compare loaves B and D in Figure 11.)

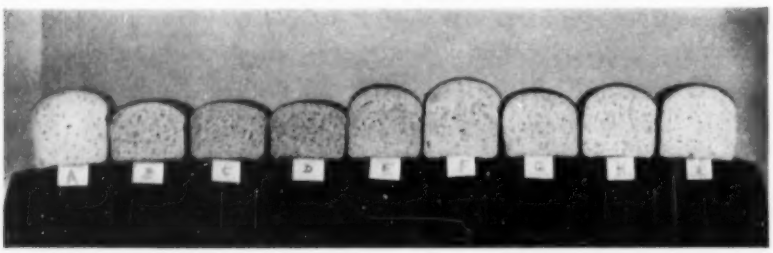

Fig. 11.

Loaf A, unbleached flour, control.
Loaf B, same as A plus 2% natural wheat germ.
Loaf C, same as A plus 2% ether and alcohol extracted wheat germ.
Loaf D, same as A plus 2% heated wheat germ (16 hours at 100° C.).
Loaf E, same as A plus cold pressed germ oil (equivalent to 2% germ).
Loaf F, same as B plus .0015% $KBrO_3$.
Loaf G, same as B plus 3 g. NCl_3 per barrel (germ added *after* bleach).
Loaf H, same as B plus 3 g. NCl_3 per barrel (germ and flour bleached together).
Loaf I, same as B plus 9 g. NCl_3 per barrel (germ and flour bleached together).

The detrimental effect of papain on the baking quality of flour (see Figure 1) and also the improving effect of $KBrO_3$ and NCl_3 will be recalled. A similar effect in the case of germ addition and the beneficial effect of both these improving agents are to be noted from Figure 11. This similarity is striking if one compares the loaves, indicated by D and E in Figure 1 with those indicated by B and F in Figure 11. No doubt similar causes produce similar results, and it is believed safe

to conclude that wheat germ contains a relatively large amount of proteolytic enzyme of a similar character to papain.

EXPERIMENT X: A series of bakes was conducted with various amounts of papain and wheat germ in order to compare their relative proteolytic effect. From this series of bakes the loaves containing papain and germ were compared and are shown as Figure 12.

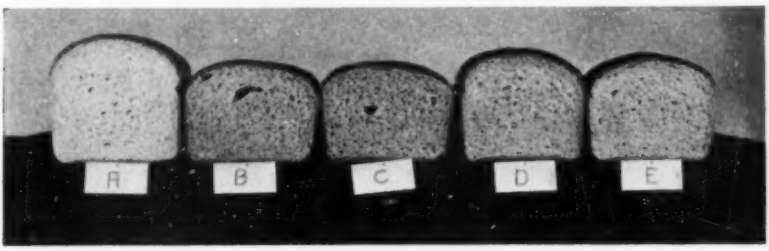

Fig. 12.

Loaf A, control flour.
Loaf B, same as A plus .0028% papain.
Loaf C, same as A plus 2% wheat germ.
Loaf D, same as A plus .0017% papain.
Loaf E, same as A plus 1.5% wheat germ.

This experiment would indicate that wheat germ has a potency expressed in terms of papain (Merck) of approximately .14%.

EXPERIMENT XI: In this experiment the relative proteolytic effect of barley-malt sprouts, wheat germ, and papain was compared. The

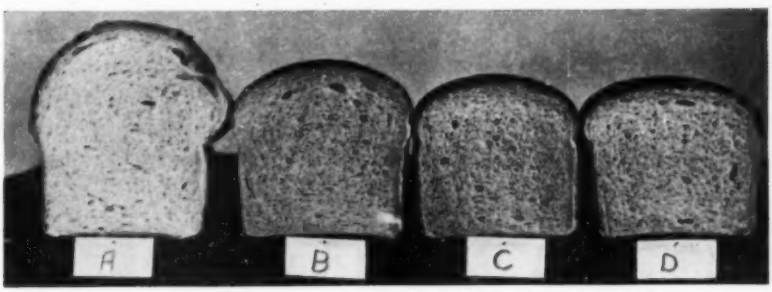

Fig. 13.

Loaf A, control flour.
Loaf B, ½% wheat germ.
Loaf C, ½% barley malt sprouts.
Loaf D, .0017% papain.

treatment and effect of these materials on the resulting loaves are shown as Figure 13.

Apparently barley-malt sprouts are of considerably higher proteolytic potency than wheat germ and which can be roughly expressed as 0.33% in terms of papain (Merck).

EXPERIMENT XII: In this experiment the modified gluten quality test was used to study the effect of the addition of alcohol-ether extracted wheat germ, unextracted germ, and malt sprouts on a standard flour, as indicated by the relative consistency of the washed-out gluten. The organization of the tests and the appearance of the resulting glutens are shown as Figure 14.

Fig. 14.

Gluten No. 1, control flour.
Gluten No. 2, plus ½% unextracted wheat germ.
Gluten No. 3, plus ½% alcohol and ether extracted germ.
Gluten No. 4, plus ½% barley-malt sprouts.

The relative flatness of the gluten balls, indicated by their height and diameter, clearly shows differences in consistency, due mainly to proteolytic action.

EXPERIMENT XIII: The following experiment deals with the effect of heat on the proteolytic enzymes in wheat germ and papain.

It was found that dry heat up to 100° C. for a prolonged time does not materially affect the activity of the proteolytic enzymes in either wheat germ or papain.

The experiments with heated wheat germ and papain in flour, mentioned in earlier parts of this paper, were made with these substances after they were left to dry at 100° C. and it has been shown that no material reduction in their proteolytic activity was observed, as may be noted from comparative baking tests.

Kent-Jones (1927) in his book "Modern Cereal Chemistry" emphasizes the fact that the improving effect of heat treatment depends to a great extent on the moisture content of the flour or wheat, as the case may be.

Geddes (1929) makes the following statement: "The experiments reported in this paper seem to offer convincing evidence that the improvement in baking quality induced by the heat-treatment of straight-grade flour is to be ascribed to changes in the germ constituents."

Geddes' statement is agreed to up to this point but we believe his subsequent conclusions, in which he absolved the proteolytic enzymes in the wheat germ from the blame for the detrimental effect on the baking quality of the flour, are erroneous.

It is contended that the reduction of proteolytic action in the flour caused by the heat treatment under favorable moisture conditions can adequately account for the improvement in the baking quality of the heat-treated flour. The reason for this statement is based on experiments, the results of which are shown in Figures 15 and 16.

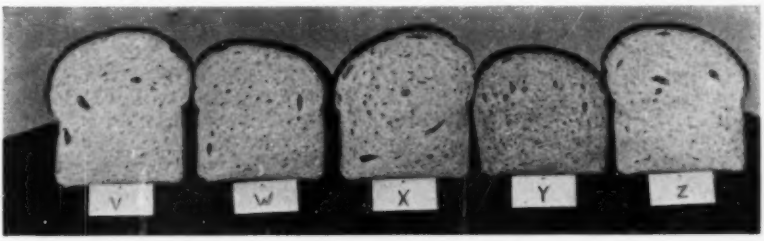

Fig. 15.

Loaf V, control flour.
Loaf W, treated with ½% fresh wheat germ.
Loaf X, treated with ½% wheat germ heated in the presence of moisture.
Loaf Y, treated with .0017% unheated papain.
Loaf Z, treated with .0017% papain, heated in the presence of moisture.

Here again it is to be noted that the proteolytic enzymes of wheat germ and those of papain behave very similarly.

The treatment here referred to consisted in exposing the wheat germ and the papain to steam at 100° C. for a period of 2 hours. Apparently these conditions are sufficient to destroy all proteolytic action of either germ or papain as they normally affect baking quality.

In the heat treatment of wheat and flour, according to Kent-Jones' patents, BP 228.830 and BP 228.841, a temperature of 100° C. would naturally be out of the question. This temperature was used only to demonstrate the difference in the effect of dry and moist heat by calling attention to the difference in the baking results here obtained as compared with those reported earlier in this paper where the result of dry heat up to 100° C. was zero.

In a final experiment an attempt will be made to show that the Kent-Jones' patents, as indicated above, are in reality based on the effect of heat and moisture on the partial inhibition of proteolytic action in flour, similar to that of added flour improvers.

EXPERIMENT XIV: A calculated amount of wheat germ in one case and a calculated amount of papain in the other were each mixed with approximately 35 g. of flour of a natural moisture content of about 13%. These mixtures were transferred to test tubes, which then were sealed tightly to prevent moisture losses. The tubes were then immersed in a water bath at the temperature and for the times specified for a certain flour moisture content, according to Kent-Jones' "Graph Showing

Relationship of Moisture in Wheat to Temperature, Required for Proper Treatment, Time Fixed 1¼ Hours." ("Modern Cereal Chemistry," page 279, 2nd edition.)

In this particular experiment 13% moisture flour-germ and flour-papain mixtures were used and held at a temperature of 72° C. for 1¼ hours.

The contents of the test tubes weighing about 35 g. were then mixed with a flour of good baking quality in such a way that the resulting mixtures contained ½% wheat germ and .0017% papain.

These flours were then baked and compared with flour mixtures containing the same basic flour as used in this test but to which in one case ½% fresh wheat germ had been added and in the other .0017% unheated papain.

The results of these bakes are shown as Figure 16.

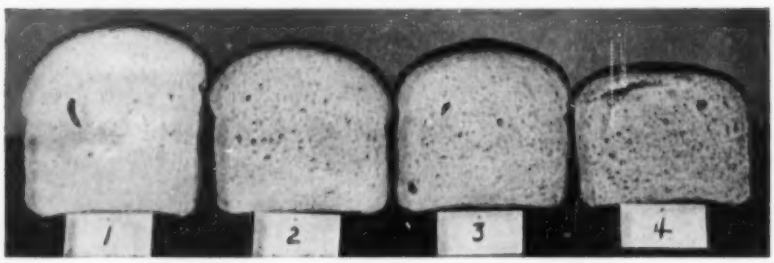

Fig. 16.
Loaf 1, flour plus ½% heat-treated wheat germ.
Loaf 2, flour plus ½% untreated wheat germ.
Loaf 3, flour plus .0017% heat-treated papain.
Loaf 4, flour plus .0017% untreated papain.

Evidently the proteolytic activity of both wheat germ and papain has been considerably weakened by the effect of moisture and heat, within the specification of the Kent-Jones patents, mentioned in this paper. From this it is apparent that flour improvement by heat treatment can be explained by the reduction of proteolytic activity in the same way as the improvement by means of flour improvers. It is also a well known fact that flour improvement by heat treatment is most successful with flours which respond well to chemical flour improvers.

Conclusions

Whether all questions regarding responsiveness to improvers can be attributed to the relative proteolytic action in flour remains to be further investigated. A satisfactory explanation for the fact that clears react better to improvers than patents in that the former have a higher germ content is available.

Why Southwestern and Canadian flours as a rule react better to chemical flour improvers than Northwestern flours requires further investigation. The variation in the behavior of these classes of flours may be due either to differences in proteolytic quantity or to differences in the susceptibility of the relative glutens to proteolytic action.

It has been observed that the response to improvers is generally greater with high gluten flours than with low gluten flours. This may be explained by the fact that an equal amount of proteolytic enzyme affects a larger quantity of gluten relatively more than a smaller quantity. For the same reason the inhibition of proteolytic action by improvers will be relatively more pronounced with high gluten than with low gluten flours.

An optimum condition for proteolytic action in the dough is absolutely necessary for best baking results. If through overbleaching or overtreatment with chemical improvers too much of the proteolytic enzymes have been destroyed, tough and unyielding doughs will be obtained, resulting in unsatisfactory bread.

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THE EFFECT OF SUCROSE AND MALTOSE UPON ACID AND GAS PRODUCTION IN DOUGHS

J. H. LANNING

Continental Baking Company, Jamaica, New York

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Introduction

While conducting some experiments with a sponge-dough baking procedure, it was noticed that sponges containing sucrose decreased in pH value at a greater rate than those to which no sugar had been added. From this observation it was believed that the increase in acid content during fermentation might be dependent to some extent upon the kind of sugar present, and since the natural sugar formed in the dough by diastatic action is maltose, this sugar would predominate in the dough to which no sucrose had been added. Proceeding from these deductions, experiments were made to determine how doughs containing maltose, sucrose, or a mixture of these sugars compared to no-sugar doughs in regard to acid and gas development, and also to find out which of the sugars was first utilized during fermentation.

Experimental

Glass electrode.—The change in pH value of the fermenting doughs was followed by means of the glass electrode. Since this is a relatively new method of determining the pH value of a dough, a detailed description of the glass electrode assembly is given. An electrode similar to the Hughes type and described by Britton (1929) was used. It was made by blowing a glass bulb about 21 mm. in diameter on the end of a piece of 9 mm. Corning 015 glass tubing. The bulb was filled with N/10 HCl with which electrical contact was made by a small calomel cell built in a glass tube as described by Britton, and which was inserted into the electrode and then sealed in place with sealing wax. The shank of the electrode was coated with paraffin to prevent a film of water from forming on it.

The glass electrode was used in conjunction with a calomel cell filled with a saturated solution of KCl, and both were supported on a block of sulphur. The lower half of a medium sized test tube was inserted in the mould when the sulphur block was cast to serve as a support for the calomel cell. A piece of glass tubing was put in the block to serve as a support for the glass electrode. Another piece of

tubing large enough to slip over the piece in the block was then taken and to one end a spring clothes pin was tied securely in place by binding it with linen thread. To make it more secure the binding was thickly coated with sealing wax. The electrode could then be held firmly by means of the clothes pin and could be swung into any position desired. All the tubes as well as the top of the sulphur block were given a heavy coating of paraffin to prevent electrical leakage. The manner in which this apparatus was assembled is shown in Figure 1.

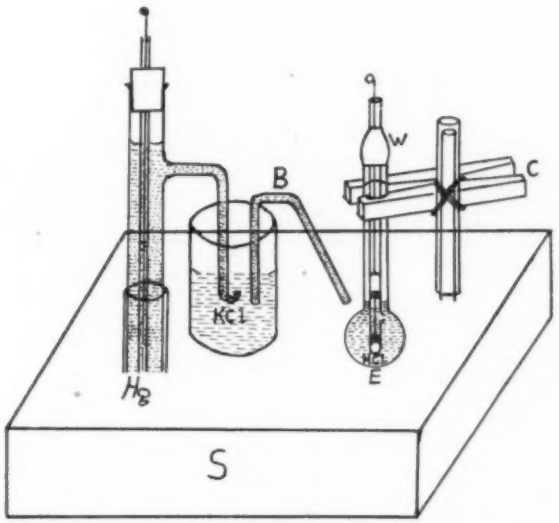

Fig. 1. Glass electrode equipment.

The E.M.F. measurements were made with a Type K potentiometer used with a vacuum tube amplifier as null point indicator. The amplifier circuit was essentially the same as that described by Ellis and Kiel (1933). The more important parts of the circuit and also an electrode suitable for insertion in a dough were described by Landis (1934).

Method for determining pH.—When making a determination, about 20 g. of dough were flattened out between the fingers and then wrapped around the bulb which had previously been wet with distilled water. It was necessary to cover the bulb completely with dough. The dough-covered electrode was allowed to rest on a small watch glass. Contact between the dough and the calomel electrode was made by means of a saturated KCl agar bridge. The sulphur block with its various parts was placed in a small tin-covered wooden cabinet to shield it from external electrical interference. The electrode was frequently checked by means of buffer solutions.

Gas measuring device.—The gas measuring apparatus consisted of three desiccators with side openings which were mounted in an 80° F. constant temperature cabinet and connected with 2000 cc. gas bottles filled with a saturated solution of sodium chloride which was slightly acidified. Leveling bottles were provided to bring the gas to atmospheric pressure. Each determination was made using a 500-g. portion of dough which was placed in a pan, the pan and dough then being inserted in the desiccator. The amount of gas evolved was later calculated to cubic centimeters per 100 g. of dough.

Dough formulas.—To determine how the pH values and the amount of gas produced varied with the sugar used, doughs were made according to the following formulas:

Dough Formulas

Ingredients	No-Sugar	5% Sucrose	5% Maltose	2½% Sucrose 2½% Maltose
	grams	*grams*	*grams*	*grams*
Flour (15% M.B.)	360	360	360	360
Water	227	227	227	227
Yeast	11	11	11	11
Arkady	1.6	1.6	1.6	1.6
Sucrose	—	18	—	9
Maltose, monohydrate	—	—	19[1]	9.5[1]

[1] Equivalent to 18 and 9 g. of anhydrous maltose.

Results

pH and gas measurement.—These doughs were fermented for 7 hours at 80° F. and measurements of their pH values were made at one hour intervals. Gas measurements of the first 3 doughs were also made at one-hour intervals. The results are shown in Tables I and II, and Figures 2 and 3.

TABLE I

EFFECT OF SUCROSE AND MALTOSE UPON THE PH VALUES OF FERMENTING DOUGHS

Fermentation time	Sugars used in dough			
	No-Sugar	5% Sucrose	5% Maltose	2½% Sucrose 2½% Maltose
Hours	*pH*	*pH*	*pH*	*pH*
0	5.53	5.59	5.58	5.54
1	5.29	5.20	5.28	5.27
2	5.25	5.03	5.19	5.06
3	5.05	4.87	5.04	4.91
4	4.99	4.77	4.92	4.86
5	4.95	4.69	4.84	4.82
6	4.94	4.67	4.79	4.81
7	4.94	4.68	4.79	4.81

TABLE II

EFFECT OF SUCROSE AND MALTOSE UPON GAS PRODUCTION OF FERMENTING DOUGHS

Fermentation time	Gas per 100 g. of dough		
	No-Sugar	5% Sucrose	5% Maltose
Hours	*Cc.*	*Cc.*	*Cc.*
1	140	132	138
2	333	364	325
3	561	605	546
4	806	865	829
5	896	1084	1120
6	952	1243	1333
7	998	1386	1482

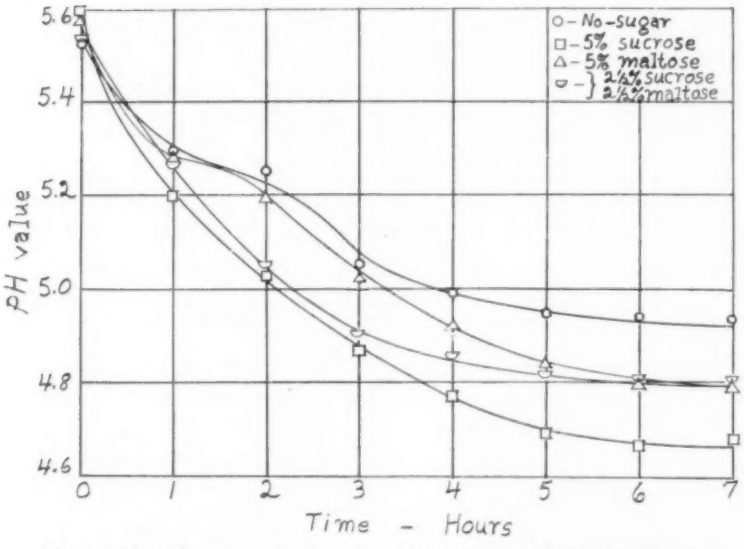

Fig. 2. Effect of sucrose and maltose upon the pH values of fermenting doughs.

The data of Table I show that doughs containing sucrose increase more rapidly in acidity during the early part of the fermentation than those containing maltose. If it is assumed that the acid formed is derived during the fermentation of sucrose, then it would appear that this sugar was first fermented from the dough.

Before sucrose is utilized by the yeast, it is converted to invert sugar. Before maltose is fermented, it is converted to dextrose. It appeared possible that the yeast might invert sucrose at a faster rate than it could utilize the sugars so formed, while in the case of maltose the change to dextrose might take place at a slower rate. If such a condition should exist, the exhaustion of the sucrose could readily be shown by forming osazones of the invert sugars at various periods

throughout the fermentation. The presence of maltose as such could also be determined readily by osazone formation since its osazone, when viewed under the microscope, is very different from those of the first mentioned sugars.

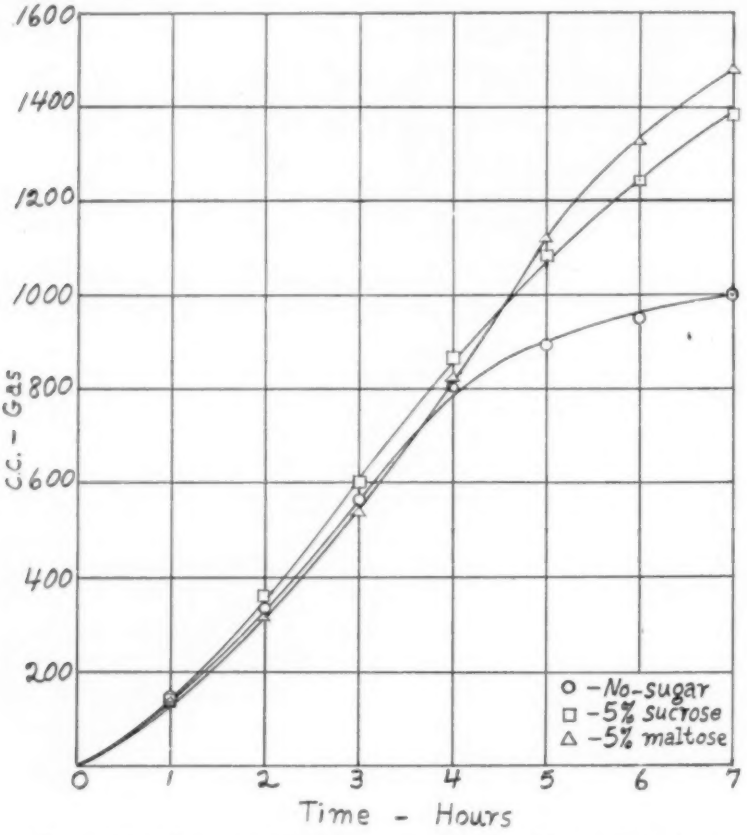

Fig. 3. Effect of sucrose and maltose upon gas production of fermenting doughs.

Osazone tests.—Osazone tests were conducted according to the general method, outlined and discussed by Brown (1912), which was rearranged in the following manner to suit our purpose: Approximately 15 g. of dough were macerated in a mortar with about 25 cc. of water. The liquid was then poured off and centrifuged. To about ½ cc. of phenylhydrazine, in a test tube, several drops of glacial acetic acid were added. About 10 cc. of the centrifuged extract were added to the phenylhydrazine-acetic acid mixture and the test tube placed in a boiling water bath for 40 minutes. When dextrose or levulose was present, osazones formed from them and crystallized from the hot solution. The maltosazones crystallized only after the solution had

been allowed to cool. The test was confirmed by a microscopic examination of the crystals.

The osazone reaction was applied, as a qualitative test for sugars, to fermenting doughs at one-hour intervals. The findings are shown in Table III.

TABLE III

OSAZONE FORMATION FROM FERMENTING DOUGHS CONTAINING SUCROSE AND MALTOSE

Fermentation time	Sugars used					
	5% Sucrose		5% Maltose		2½% Sucrose 2½% Maltose	
	D	M	D	M	D	M
Hours						
1	+	sl.	v.s.	+	+	+
2	+	sl.	v.s.	+	sl.	+
3	+	+	v.s.	+	sl.	+
4	+	+	v.s.	+	v.s.	+
5	v.s.	+	−	+	−	+
6	−	−	−	−	−	sl.

D = dextorse or levulose. (These form the same osazone.)
M = maltose.
+ = positive osazone crystallization.
sl. = slightly positive osazone crystallization.
v.s. = very slightly positive osazone crystallization.
− = negative osazone crystallization.

It is possible that some sucrose inversion did occur in these tests but it was noted that when an abundance of sugar was present in the sucrose doughs, the osazones of the invert sugars would crystallize abundantly soon after being placed in the boiling water bath. Tests made using sucrose solutions also gave positive tests, but the time before crystallization took place was somewhat longer. From these observations, it would appear that much of the invert sugar in the dough solutions was present before the solutions were placed in the boiling water bath.

The experiments were repeated using another type of bakers' yeast. The results were so similar in trend to those first obtained that it is not necessary to show them in the form of curves. The data are given in Tables IV, V, and VI.

Conclusions

The acidity curves in Figure 2 show a more rapid decline in pH value for the doughs containing only added sucrose than from those containing the added maltose. They also show that the greater por-

tion of this decline occurred during the first three or four hours of fermentation.

The osazone tests showed that the sucrose was more readily fermented from the dough than the maltose.

TABLE IV

EFFECT OF SUCROSE AND MALTOSE UPON THE pH VALUES OF FERMENTING DOUGHS

Fermentation time	Sugars used in dough			
	No-Sugar	5% Sucrose	5% Maltose	2½% Sucrose 2½% Maltose
Hours	*pH*	*pH*	*pH*	*pH*
0	5.66	5.72	5.67	5.58
1	5.39	5.35	5.37	5.34
2	5.28	5.15	5.22	5.10
3	5.13	4.97	5.06	4.96
4	5.00	4.81	4.92	4.89
5	5.01	4.76	4.83	4.85
6	4.96	4.76	4.85	4.91
7	4.99	4.75	4.82	4.85

TABLE V

EFFECT OF SUCROSE AND MALTOSE UPON GAS PRODUCTION OF FERMENTING DOUGHS

Fermentation time	Gas per 100 g. of dough		
	No-Sugar	5% Sucrose	5% Maltose
Hours	*Cc.*	*Cc.*	*Cc.*
1	111	120	130
2	274	307	301
3	498	532	518
4	738	766	784
5	837	971	1017
6	902	1146	1215
7	946	1290	1406

TABLE VI

OSAZONE FORMATION FROM FERMENTING DOUGHS CONTAINING SUCROSE AND MALTOSE

Fermentation time	Sugars used					
	5% Sucrose		5% Maltose		2½% Sucrose 2½% Maltose	
	D	M	D	M	D	M
Hours						
1	+	+	sl.	+	+	+
2	+	+	−	+	+	+
3	+	+	−	+	+	+
4	−	+	−	+	−	+
5	−	+	−	+	−	+
6	−	+	−	+	−	−

The curves in Figure 3 show that a greater amount of gas was produced from the maltose than from the sucrose doughs. Since all the doughs, except the no-sugar doughs, had the same sugar concentration, the gassing tests indicate that a portion of the sucrose was used by the yeast for a purpose other than gas production. This, according to the data obtained, was for the production of acid. Both types of bakers' yeast gave results of a similar trend.

An examination of the acidity curve for the no-sugar sponge, represented in Figure 2, shows that there is a rapid increase in acidity at the beginning of the fermentation. This increase then rapidly subsides and the curve becomes flat. Later, it again begins to rise. This is probably due to the fermentation of the sucrose naturally present in the flour. Acid is rapidly produced until this sugar has been utilized, after which the yeast is dependent entirely on the maltose produced by diastatic activity. It appears that a period of time then elapses before the yeast is able to form an accumulation of acid from the maltose at a rapid rate.

Summary

Acidity was developed more rapidly in doughs containing sucrose than in those containing maltose.

By forming osazones of the sugars that were in the doughs, it was found that sucrose was fermented from the doughs in preference to maltose.

Practically the same results were obtained with two different types of commercial bakers' yeast.

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OVEN SPRING OF DOUGH AS INFLUENCED BY SUGAR, SALT, AND YEAST [1]

W. O. WHITCOMB

Montana Agricultural Experiment Station, Bozeman, Montana

(Read at the Annual Meeting, June 1936)

The factors influencing the oven spring of dough are of importance in the experimental baking test. This paper is limited to a study of the influence on oven spring of varying amounts of sugar, salt, and yeast, taken separately, when the other ingredients and the methods of baking are held constant.

Harrel (1926) studied the baking properties of dough as influenced by 9 variables and noted the effect on the properties of the bread. Variation in fermentation period gave the most pronounced results in oven spring. This oven spring was stated in terms of good, fair, and poor. Micka and Child (1928) found that sugar, salt, and lard decreased the absorption of flour, while yeast increased it to a lesser extent. Merritt, Blish, and Sandstedt (1932) showed the possibility of eliminating sugar from ingredients making up the dough and reducing the fermentation period by one-half. Sandstedt and Blish (1933) recommended the use of 1% sugar in doughs from experimentally milled flours because of diastatic deficiencies when using the so-called "short method." Larmour and Brockington (1933) showed that sugarless dough containing 0.001% bromate and 0.5% diastatic malt was a suitable formula for determining strength of flour. In 1934, Larmour and Brockington suggested a formula containing 6% sugar, 5% yeast, and 0.001% $KBrO_3$.

Materials and Methods

Seven flours of varying characteristics were included in the study. Chemical analyses of these flours are shown in Table I. Five of these flours were blends from remnants of test samples which had been milled from lots of wheat ranging from 10 to 60 bushels. These 5 samples somewhat resembled commercially-milled flours; while 1 sample of the group was experimentally milled; and the 7th sample was a bleached commercial flour. Judged by their ash content, all these flours would be classed as patents. Their protein content, with two exceptions, was above 12%. The sugar and salt used were of

[1] Contribution from Montana State College, Agricultural Experiment Station. Paper No. 72, Journal Series.

good commercial grade. Fleischmann's compressed yeast, which was obtained fresh every third day and kept near ice, was used.

TABLE I

DESCRIPTION OF FLOURS

Flour No.	Wheat from which milled [1,2,3]	Age of flour	Protein [4]	Ash [4]	Absorption [4]	Diastatic activity [5]
		Months	%	%	%	
Check	Commercial blend	2	13.05	.42	59	
1	Blend of 1934 Marquis	14	15.25	.38	59	192
2	Blend of 1934 Thatcher	14	15.70	.38	61	200
3	Blend of 1935 hard red spring	4	14.20	.36	56	375
4	1935 Marquis		12.90	.39	56	108
5	Blend of 1934 hard red winter	14	10.80	.38	61	284
6	Blend of 1935 hard red winter	4	13.00	.34	56	208
7	Commercial blend	4	11.15	.41	59	234

[1] Check flour and No. 7 were bleached, other flours were not bleached.
[2] Flours 1, 2, 3, 5, and 6 were blends of remnants of test samples milled in 10 to 60 bushel lots and resembled somewhat commercially milled flours.
[3] Flour No. 4 milled on experimental mill 3 days before series of baking tests were begun.
[4] Basis 15.0% moisture.
[5] Milligrams of maltose per 10g. of flour in 1 hour at 30° C.

The Standard A. A. C. C. Baking Test method as described in Cereal Laboratory Methods, Third Edition (1935), was used with the following exceptions:

(1) *Basic Formula:* 3% sugar was used instead of 2.5%.

(2) *Standard Procedure:* A proofing and fermentation cabinet as described by Whitcomb (1934) was used and the temperature of the doughs was carefully controlled. The punching of the dough was done by passing it between canvas-covered sheeting rolls running ¼-inch apart and driven with a motor. The panning was done by running the dough through these same rolls and tightly rolling the resulting strip of dough on canvas belting as described in the handbook.

The oven spring was measured by placing the pan of dough on a base just before putting it in the oven, and sliding a projecting arm down to the topmost point of the dough. The distance from the base to the surface of the dough was then read on the standard which supported the arm. When the baked loaf was taken from the oven, the height was measured in the same manner as that of the dough. The difference in these 2 measurements was considered as the oven spring.

A check dough was baked in triplicate with each day's tests. As a measure of accuracy in determining the oven spring, 24 check doughs were baked at the beginning of the series and a like number were

baked at the conclusion. The average oven spring of those baked at the beginning was 1.2 cm., and the range was from 0.8 to 1.5 cm. Of the 24 doughs, 3 were 0.8 cm., 7 were 1.0 cm., 8 were 1.3 cm., and 6 were 1.5 cm. The average oven spring of those baked at the conclusion was 1.1 cm., with a range from 1.0 cm. to 1.5 cm. Of these, 13 were 1.0 cm., 9 were 1.3 cm., and 2 were 1.5 cm. These tests would indicate that an accuracy of 0.5 cm. might be expected.

Results

The different flours showed a marked response to the use of varying amounts of sugar, salt, and yeast as judged by the oven spring of the dough.

Sugar.—As shown in Table II, the doughs made with flour No. 7

TABLE II

SUGAR AS INFLUENCING FACTOR IN OVEN SPRING OF DOUGH FROM DIFFERENT FLOURS

Sugar	Oven spring of flours							
	Check	1	2	3	4	5	6	7
%	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
0	1.4	2.3	2.9	1.3	1.5	0.6	0.5	2.8
1	1.4	2.4	3.7	3.6	2.5	0.8	1.4	1.4
2	1.4	1.3	2.1	2.0	1.3	0.6	0.5	1.4
3	1.0	1.6	1.5	1.8	0.5	0.7	0.3	0.8
4	1.0	1.3	1.7	2.5	0.8	0.5	0.6	1.0
5	1.3	1.3	1.6	1.9	0.9	0.5	0.8	1.1

with no sugar had the greatest oven spring. Doughs made with flours No. 2, 3, 4, and 6 had the greatest oven spring with 1% sugar, whereas the doughs made with flour No. 1 had much the same oven spring with no sugar and 1% sugar. The dough made with flour No. 5 was but little influenced by varying the sugar from none to 5%. When 5% sugar was used, all doughs were sticky during mixing, punching, and panning. The top crusts of all the loaves made from the 5% sugar doughs, except from flour No. 4, were too dark, which indicated a good supply of sugar during baking, but which was not reflected in an increased oven spring.

Salt.—At least 1% salt seemed necessary for maximum oven spring with doughs made from flours No. 1, 3, and 7. According to the data given in Table III, the doughs made with the other flours needed 1.5 to 2.0% salt to produce maximum oven spring. As much as 2.5% salt was not injurious to oven spring of the doughs when using any of the flours. An appreciable decrease in oven spring was caused when using 3.0% salt in the doughs made from flours No. 1, 3, and 7. The elimination of salt caused all the doughs to be sticky, but not slack.

TABLE III

SALT AS INFLUENCING FACTOR IN OVEN SPRING OF DOUGH FROM DIFFERENT FLOURS

	Oven spring of flours							
Salt	Check	1	2	3	4	5	6	7
%	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
0	1.4	0.4	0.5	1.2	0.7	0.2	0.2	0.7
1.0	1.0	1.6	1.5	1.8	0.5	0.7	0.3	0.8
1.5	1.0	1.3	1.5	1.8	0.9	1.2	1.0	1.0
2.0	1.2	1.7	2.1	1.4	1.3	1.3	1.3	1.6
2.5	1.2	1.3	1.9	2.0	0.9	1.3	1.0	1.4
3.0	1.0	0.8	1.7	1.3	1.0	1.3	1.6	1.1

This condition was more pronounced at mixing time and during the first punch. The doughs were not sticky when panned. The use of 3.0% salt caused the doughs to be sticky when mixed but they were not sticky after mixing. When using flours No. 1, 2, 3, and 6, yeast action was retarded by the use of 3.0% salt as shown by the slow rising of the dough during fermentation and the deep brown color of the top crusts of the loaves.

Yeast.—As shown in Table IV, the doughs made from 3 of the

TABLE IV

YEAST AS INFLUENCING FACTOR IN OVEN SPRING OF DOUGH FROM DIFFERENT FLOURS

	Oven spring of flours							
Yeast	Check	1	2	3	4	5	6	7
%	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*	*Cm.*
1	1.1	1.9	1.8	2.9	1.1	1.3	0.7	2.2
2	1.0	1.2	1.9	2.3	0.8	1.0	0.8	1.1
3	1.0	1.6	1.5	1.8	0.5	0.7	0.3	0.8
4	1.2	1.8	2.3	1.4	1.3	0.6	0.6	1.0
5	1.0	2.9	3.5	1.6	2.0	0.6	1.0	1.6

flours had better oven spring with 1% yeast than they did with more yeast. Three other flours gave the best oven spring with 5% yeast. Doughs from flour No. 6 did not respond to increasing amounts of yeast. The use of 5% yeast caused the doughs to be sticky when mixed but they were only slightly sticky when punched. These doughs all fell before the first punch, raised well for the second punch, but did not raise well in the pan. When 5% sugar was used, the crust color of the bread baked from flour No. 3 was too dark. This indicates a good supply of sugar as the dough went into the oven. With bread baked from flours No. 1, 2, 4, 6, and 7 the crust color was too light, indicating that the sugar supply had been exhausted. The

excess of sugar in the loaf made from flour No. 3 seemed to indicate yeast exhaustion because the oven spring was less than when a smaller quantity of yeast was used. On the other hand, doughs made from flours No. 1, 2, and 4 showed an increased oven spring with 5% yeast even though the sugar was exhausted when the dough was baked.

Summary

Seven flours of varying chemical characteristics showed a wide range in oven spring of their doughs when baked with varying quantities of sugar, salt, and yeast.

One flour produced a dough which had the greatest oven spring when no sugar was used, while 4 other flours gave doughs which had the greatest oven spring with 1.0% sugar.

Abundance of sugar in dough at time of baking, as indicated by dark brown color of top crust, was not reflected in high oven spring.

With 3 flours at least 1.0% salt was needed in the dough for maximum oven spring. With the 4 other flours 1.5 to 2.0% salt was required to produce maximum oven spring.

Three flours produced doughs which had better oven spring when 1.0% yeast was used than with greater amounts. Three other flours produced doughs which gave the best oven spring with 5.0% yeast.

Increased oven spring was observed with the doughs of 3 flours when 5.0% yeast was used even though the sugar had become exhausted at the time the dough was baked.

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CAKE-MAKING QUALITY OF EGGS AS RELATED TO SOME FACTORS IN EGG PRODUCTION

FLORANCE B. KING, ELIZABETH FULLER WHITEMAN, and W. G. ROSE

Bureau of Home Economics, United States Department of Agriculture, Washington, D. C.

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This paper reports a continuation of the cooperative study of egg quality which is under way in the Bureaus of Animal Industry, Chemistry and Soils, and Home Economics of the United States Department of Agriculture. This part of the study was planned to show the effect, if any, of the diet of the hen, and of the season of the year or the laying cycle of the hen, on the properties of the eggs and on cakes in which these eggs were used. In connection with this study the data on carbon dioxide content and pH of these eggs were used also to compare with similar measurements on stored eggs.

In a paper reporting the earlier part of the present investigation, King, Morris, and Whiteman (1936) described the methods and apparatus used in studying the leavening property of eggs, measured quantitatively by a determination of several physical properties of sponge cakes. The measurements included the weight, volume, tensile strength, and compressibility for each of six cakes baked from each lot of eggs received. The specific gravity and pH of the cake batter were also determined. Physical and chemical measurements made on a representative sample of the eggs used in making the cakes included: Total solids of the white and yolk; pH of the white, the yolk and the magma of white and yolk mixed in the same ratio as that in which the white and yolk were used in the sponge cake batter; the viscosity of this magma, and the carbon dioxide content of the white and yolk.

The effect of diet on egg composition has been studied by Titus, Byerly, and Ellis (1933). Different rations were fed to nine groups of hens. At intervals during the experiment, all the eggs laid on certain days were saved for chemical analysis. The constituents determined included total solids, protein of white and yolk, and fat (ether extract) of the yolk. It was shown that the effect of the diet of the hen on these constituents of the egg was not great.

The present writers are not aware of any studies on the effect of

the diet of the hen on the properties of eggs in relation to their use in food preparation.

Methods and Material

In both this and the previous investigation, the eggs were produced by the Bureau of Animal Industry at the Beltsville Research Center of the United States Department of Agriculture.[1]

The eggs were obtained from five groups of Barred rock-Rhode Island red crossbred hens which had been fed different rations as indicated in Table I.

TABLE I

RATIONS FED HENS PRODUCING EGGS USED IN THESE EXPERIMENTS

	Group I	Group II	Group III	Group IV	Group V
ingredients	%	%	%	%	%
Basal feed mixture [1]	90.80	70.89	71.00	71.00	73.46
Ground limestone	3.37	3.30	3.32	3.32	3.35
Steamed bonemeal	4.83	4.81	4.68	4.68	2.19
Sodium sulfate (anhydrous)	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Soybean oil cake meal	—	10.00	10.00	—	—
Peanut oil cake meal	—	10.00	—	10.00	—
Linseed oil cake meal	—	—	10.00	10.00	—
Fish meal, meat meal, and dried buttermilk	—	—	—	—	20.00

[1] Basal feed mixture consists of: yellow corn meal, 52.63%; wheat bran, 25.79%; rolled oats, 15.79%; alfalfa leaf meal, 5.79%.

The eggs were collected and dipped in mineral oil saturated with carbon dioxide, in the way described in the earlier paper. In the present study, however, the eggs were collected during a two to four day period, and held at a temperature of 40–60° F. They were thus two to five days old at the time they were used for the cakes and for the chemical and physical measurements.

In addition to the measurements for specific volume, compressibility, and tensile strength of the cakes, a measurement of elasticity was included, as an additional check on the softness of the cake. The same portion of cake which was measured for compressibility was used in determining the elasticity. After the compressibility value had been recorded, the weight and disk of the penetrometer were removed from the test piece for one minute, then the disk was again allowed to come to rest on the cake for 30 seconds, the set-screw holding the disk was tightened, and the height of the disk was read on the scale which is engraved on the post holding the disk. The difference between this reading and the height of the disk when the cube of cake was compressed by a 300 g. weight (see compressibility measurement in the earlier paper) gives the recovery or elasticity.

[1] The authors wish to express their appreciation to Dr. H. W. Titus, Mr. Ralph B. Nesbit, and their associates at the National Agriculture Research Center for their cooperation in supplying the eggs used in this investigation.

Results

Table II gives the results of physical and chemical determinations made on the eggs the same day the cakes were baked. Table III records the results of the measurements made on the cake batter and on the finished cakes. The values on the finished cakes are averages of six cakes for each baking.

TABLE II

INDIVIDUAL AND AVERAGE VALUES OF PHYSICAL AND CHEMICAL MEASUREMENTS ON THE EGGS

Date measurement made	Group of hens	% total solids			pH			Carbon dioxide mg. in 100 cc.		Viscosity of magma
		White	Yolk	Magma	White	Yolk	Magma	White	Yolk	Time of outflow, seconds
12/18/34	I	12.82	51.14	27.19	8.25	6.07	7.23	230.9	6.68	37.2
1/ 8/35	I	12.39	52.60	27.47	8.08	5.98	7.18	220.6	7.45	36.0
1/22/35	I	12.58	52.41	27.52	8.02	6.06	7.17	224.6	8.73	35.5
3/19/35	I	12.18	52.18	27.18	7.89	6.15	7.19	237.4	9.32	34.2
4/ 2/35	I	12.05	52.25	27.12	8.05	6.05	7.19	240.1	11.98	34.3
4/16/35	I	12.02	52.21	27.09	8.20	6.11	7.21	216.9	6.93	34.8
5/ 7/35	I	11.55	52.38	26.86	8.02	6.08	7.20	227.5	10.95	33.9
5/21/35	I	11.62	51.80	26.71	8.04	6.06	7.18	168.8	10.97	34.6
6/ 4/35	I	11.52	52.11	26.74	7.85	6.03	7.08	197.4	6.42	34.6
6/13/35	I	11.96	51.90	26.94	7.99	6.07	7.13	212.5	8.79	33.8
Average	I	12.07	52.10	27.08	8.04	6.07	7.18	217.7	8.82	34.9
12/ 6/34	II	12.69	50.99	27.04	8.70	6.03	7.34	209.2	8.55	43.2
1/10/35	II	12.22	51.66	27.01	8.12	6.05	7.20	232.5	7.45	37.3
1/24/35	II	12.00	52.12	27.04	8.11	6.11	7.22	251.8	9.55	36.9
2/ 7/35	II	11.94	52.16	27.02	8.04	6.09	7.19	202.5	8.84	35.8
3/21/35	II	12.23	52.32	27.26	8.10	6.14	7.21	231.1	7.30	34.8
4/ 4/35	II	12.04	52.32	27.14	8.23	6.03	7.17	236.4	9.38	34.8
4/18/35	II	12.04	52.06	27.04	8.15	6.09	7.21	253.1	7.29	34.8
5/ 9/35	II	12.04	52.24	27.11	8.12	6.01	7.18	242.1	7.45	34.2
5/23/35	II	11.87	51.94	26.89	8.24	6.00	7.21	184.0	4.61	34.4
6/25/35	II	11.64	52.24	26.86	7.98	6.06	7.20	176.3	8.63	34.6
Average	II	12.07	52.00	27.04	8.18	6.06	7.21	221.9	7.90	36.1
12/11/34	III	12.78	52.09	27.48	8.14	6.12	7.23	219.5	9.55	38.7
12/27/34	III	12.70	51.57	27.27	8.25	6.15	7.30	256.1	7.72	38.0
1/15/35	III	12.83	51.88	27.47	8.00	5.92	7.22	234.6	8.08	35.8
2/12/35	III	12.05	51.72	26.92	8.39	6.11	7.21	177.5	5.16	35.1
3/26/35	III	12.25	51.88	27.11	7.89	6.02	7.15	214.3	5.87	35.2
4/ 9/35	III	12.30	52.07	27.21	8.09	6.02	7.07	239.0	8.37	35.3
4/30/35	III	12.06	52.12	27.08	8.19	6.02	7.19	223.8	7.07	35.0
5/28/35	III	11.64	51.98	26.77	8.08	5.93	7.22	226.1	3.60	32.4
6/ 6/35	III	11.81	52.42	27.04	8.08	5.95	7.63	227.7	7.58	34.5
6/18/35	III	11.70	52.06	26.84	7.94	6.00	7.21	231.1	9.46	33.0
Average	III	12.21	51.98	27.12	8.10	6.02	7.24	225.0	7.25	35.3

TABLE II—(*Continued*)

Date measurement made	Group of hens	% total solids			pH			Carbon dioxide mg. in 100 cc.		Viscosity of magma
		White	Yolk	Magma	White	Yolk	Magma	White	Yolk	Time of outflow, seconds
12/13/34	IV	12.50	50.91	26.90	8.05	6.04	7.34	233.6	10.64	35.5
1/ 3/35	IV	12.06	50.95	26.64	8.30	6.10	7.36	207.0	19.32	35.9
1/17/35	IV	12.26	52.33	27.29	8.08	6.07	7.33	230.7	10.40	34.4
1/31/35	IV	12.08	52.06	27.09	8.23	6.11	7.28	231.7	8.45	34.0
2/14/35	IV	12.03	51.96	27.00	8.22	6.11	7.27	233.0	8.57	35.4
3/28/35	IV	12.40	51.93	27.22	8.05	6.08	7.17	212.4	8.43	33.9
4/11/35	IV	12.24	52.29	27.26	7.91	6.07	7.18	244.5	8.49	34.7
5/ 2/35	IV	11.87	52.06	26.94	8.15	5.99	7.24	223.4	6.70	34.9
6/20/35	IV	11.90	52.05	26.96	8.16	6.03	7.14	206.8	8.51	35.6
6/27/35	IV	11.88	51.82	26.86	8.17	6.00	7.16	202.7	7.05	34.6
Average	IV	12.12	51.84	26.92	8.13	6.06	7.25	222.6	9.65	34.9
2/20/35	V	11.99	52.19	27.06	7.96	6.09	7.16	226.7	12.17	34.0
2/26/35	V	12.04	52.30	27.14	8.05	6.06	7.11	238.6	11.05	34.9
2/28/35	V	12.10	52.24	27.15	8.05	6.08	7.19	216.7	8.73	34.7
3/12/35	V	11.78	52.23	26.95	8.09	6.07	7.22	245.1	12.10	33.7
3/14/35	V	12.37	52.76	27.48	8.06	6.06	7.20	175.3	7.68	34.4
Average	V	12.06	52.34	27.16	8.04	6.07	7.18	220.5	10.34	34.3
F from sample		3.15	1.26	1.89	1.55	1.14	2.60	6.80	2.28	1.17
F required for 5% point		5.63	2.61	5.63	2.61	2.61	5.63	5.63	2.61	2.61

Tables IV and V are set up to indicate any differences that may be attributed to the effect of the season of the year or the laying cycle of the hen. The averages for the months of December, March, and June include the results for all five groups of hens.

Discussion

Snedecor's (1934) method for determining variance between classes and within classes was used to determine whether the variation between the group means of the measurements shown in Tables II and III were enough greater than those within groups to be considered significant. *F* indicates the ratio of the larger mean square to the smaller mean square. Two values for *F* are given, one calculated from the sample and the other corresponding to the 5% point of significance. Comparison of the *F* obtained from the sample with the required *F*, indicates that the variance between groups is less than the variance within groups. Since the differences between different samples of

eggs and cakes made from the eggs of the same group of hens were greater than the differences between the averages for the five groups, it is believed that none of the differences can be attributed to the diet of the hens.

These results are in good agreement with averages reported by King, Morris, and Whiteman (1936) for composite samples of eggs from mature hens receiving several different diets, although the earlier

TABLE III

INDIVIDUAL AND AVERAGE VALUES OF MEASUREMENTS ON THE CAKES

Baking date	Group of hens	Cake batter		Finished cakes			
		pH	Specific gravity	Specific volume, cc. per g.	Tensile strength, g. per sq. cm.	Reciprocal compressibility, cm.[1]	Elasticity, cm.
12/18/34	I	5.24	.372	4.83	28.79	.79	.99
1/ 8/35	I	5.29	.378	4.86	24.26	.73	1.08
1/22/35	I	5.13	.378	4.69	23.97	.75	.99
3/19/35	I	5.28	.371	4.96	23.34	.69	1.07
4/ 2/35	I	5.28	.358	4.91	23.92	.67	1.06
4/16/35	I	5.24	.382	4.82	23.62	.73	1.03
5/ 7/35	I	5.29	.376	4.81	22.07	.76	.99
5/21/35	I	5.22	.374	4.69	23.22	.74	1.01
6/ 4/35	I	5.17	.363	4.84	24.37	.70	1.04
6/13/35	I	—	.353	4.94	20.38	.70	1.02
Average	I	5.24	.371	4.84	23.79	.73	1.03
12/ 6/34	II	5.22	.388	4.86	24.92	.71	1.08
1/10/35	II	5.18	.340	4.93	22.64	.69	1.09
1/24/35	II	5.25	.374	4.88	23.03	.76	1.03
2/ 7/35	II	5.41	.394	4.68	24.66	.78	.97
3/21/35	II	5.23	.365	4.93	24.17	.69	1.05
4/ 4/35	II	5.33	.375	4.86	24.80	.69	1.05
4/18/35	II	5.30	.369	4.94	26.32	.69	1.13
5/ 9/35	II	5.53	.374	4.82	23.56	.69	1.06
5/23/35	II	5.20	.363	4.85	26.32	.73	1.03
6/25/35	II	4.48	.342	4.89	21.30	.68	.95
Average	II	5.21	.368	4.86	24.17	.71	1.04
12/11/34	III	5.25	.402	4.69	26.41	.80	.95
12/27/34	III	5.22	.383	4.79	24.84	.78	.98
1/15/35	III	5.12	.389	4.73	25.26	.77	.99
2/12/35	III	5.69	.387	4.75	24.32	.77	.98
3/26/35	III	5.26	.370	4.93	24.84	.69	1.12
4/ 9/35	III	5.26	.400	4.76	23.76	.89	1.04
4/30/35	III	5.21	.363	4.87	24.32	.73	1.07
5/28/35	III	5.10	.370	4.85	22.92	.71	1.06
6/ 6/35	III	5.22	.361	4.86	23.22	.73	1.06
6/18/35	III	4.65	.344	4.95	24.40	.64	1.08
Average	III	5.20	.375	4.82	24.43	.75	1.03

TABLE III—(*Continued*)

Baking date	Group of hens	Cake batter		Finished cakes			
		pH	Specific gravity	Specific volume, cc. per g.	Tensile strength, g. per sq. cm.	Reciprocal compressibility, cm.[1]	Elasticity, cm.
12/13/34	IV	5.15	.373	4.89	24.30	.73	1.07
1/ 3/35	IV	5.14	.363	4.87	23.90	.70	1.03
1/17/35	IV	5.20	.372	4.86	25.25	.76	1.03
1/31/35	IV	5.21	.391	4.58	25.47	.89	.89
2/14/35	IV	5.56	.371	4.78	19.70	.74	1.05
3/28/35	IV	5.11	.323	4.84	21.99	.69	1.03
4/11/35	IV	5.30	.350	4.94	22.17	.67	1.08
5/ 2/35	IV	5.20	.342	4.93	22.37	.73	1.07
6/20/35	IV	4.81	.368	4.88	22.47	.70	1.03
6/27/35	IV	4.61	.359	4.90	21.68	.69	1.02
Average	IV	5.13	.361	4.85	22.93	.73	1.03
2/20/35	V	5.46	.367	4.90	21.54	.71	1.08
2/26/35	V	5.29	.366	4.70	24.54	.76	1.01
2/28/35	V	5.35	.391	4.85	27.23	.73	1.06
3/12/35	V	5.23	.378	4.86	24.62	.69	1.08
3/14/35	V	5.23	.361	4.88	29.73	.78	1.04
Average	V	5.31	.373	4.84	25.53	.73	1.05
F from sample		1.53	1.26	2.80	1.84	1.27	2.72
F required for 5% point		5.63	2.61	5.63	2.61	5.63	5.63

[1] Compressibility is expressed in centimeters.

study showed some differences in the measurements on pullet eggs and hen eggs.

The eggs used in this investigation, except Group V, were obtained during a period of seven months, beginning about three months after the hens started laying. Averages of the number and weights of the eggs laid by the hens in Groups I, II, III, and IV during the experimental period were essentially the same. An analysis of the data does not indicate a progressive change in any of the measurements which could be attributed to the effect of the laying cycle or the season of the year.

No relation could be established between any of the physical and chemical measurements made on the eggs and the measurements made on the cakes.

There is a relationship between the specific volume, elasticity, and compressibility of the sponge cakes, and between the pH and specific gravity of the batter from which the cakes were baked, but there is no relationship between any of these variables and the chemical or

TABLE IV

AVERAGE RESULTS OF PHYSICAL AND CHEMICAL DETERMINATIONS ON EGGS LAID DURING DECEMBER, MARCH, AND JUNE

Average for month of	Number of samples in average	% total solids			pH			Carbon dioxide, mg. per 100 cc.		Viscosity of magma
		White	Yolk	Magma	White	Yolk	Magma	White	Yolk	Time of outflow, seconds
December	5	12.70	51.34	27.18	8.28	6.08	7.29	229.9	8.63	38.5
March	6	12.20	52.22	27.20	8.01	6.09	7.19	219.3	8.45	34.4
June	7	11.77	52.05	26.89	8.01	6.02	7.22	207.8	8.06	34.4

TABLE V

AVERAGE RESULTS OF MEASUREMENTS ON THE CAKE BATTER AND FINISHED CAKES MADE WITH EGGS LAID DURING DECEMBER, MARCH, AND JUNE

Average for month of	Number of samples in average	Cake batter		Finished cakes			
		pH	Specific gravity	Specific volume, cc. per g.	Tensile strength, g. per sq. cm.	Reciprocal compressibility[1]	Elasticity, cm.
December	5	5.22	.384	4.81	25.85	.76	1.01
March	6	5.22	.358	4.90	26.60	.71	1.06
June	7	4.82[2]	.356	4.89	22.55	.69	1.03

[1] Compressibility is expressed in centimeters.
[2] This value is the average of six determinations instead of 7.

physical measurements made on the eggs. The equations expressing these relationships, and the coefficient of correlation (r) are:

$$-1/\text{compressibility} = 2.78973 - 0.42555 \text{ specific volume},$$
$$r = -.740.$$
$$\text{Elasticity} = -.89901 + 0.39974 \text{ specific volume},$$
$$r = .753.$$
$$\text{Elasticity} = 1.4490 - 0.56593\ (1/\text{compressibility}),$$
$$r = -.617.$$
$$\text{Batter specific gravity} = 0.1124 + 0.04955 \text{ batter pH},$$
$$r = .650.$$

According to the tests given by Fisher (1932) for determining the significance of an observed correlation coefficient, values of r of this magnitude could arise by chance much less than once in 100 trials,

if the correlation was zero in the universe from which the samples were drawn.

Table VI presents the carbon dioxide content and pH of individual

TABLE VI

pH AND CARBON DIOXIDE CONTENT OF OIL-DIPPED EGGS OF DIFFERENT AGES HELD AT 40–45° F. AND OF MIXED EGG WHITES OF FRESH EGGS RECEIVED AT THE SAME TIME THE EGGS WERE STORED

Hen number	Date of laying	Age of egg	pH	CO_2 per 100 cc. white	Mixture of fresh egg whites: pH	Mixture of fresh egg whites: CO_2 per 100 cc. white
		days		*mg.*		*mg.*
7466	12/ 9/34	213	8.25	200.9	8.05	233.6
7466	1/26/35	165	8.08	229.5	8.23	231.7
7466	3/24/35	108	8.04	216.7	8.05	212.4
7466	4/28/35	73	8.00	197.4	8.15	223.4
7466	6/17/35	23	8.10	172.8	8.16	206.8
7470	12/ 9/34	211	8.12	172.4	8.05	233.6
7470	1/26/35	163	7.87	226.0	8.23	231.7
7470	4/29/35	72	7.74	213.9	8.15	212.4
7470	6/18/35	34	8.41	156.2	8.16	206.8
7633	12/14/34	213	8.25	172.8	8.25	230.9
7633	3/15/35	122	8.10	216.5	7.89	237.4
7633	6/ 3/35	42	7.86	169.6	7.85	197.4

eggs that had been oil-dipped and held one to eight months at a temperature of 40–45° F. For purposes of comparison, there is included in the table the pH and carbon dioxide content of the mixed white of 15 fresh eggs laid by hens receiving the same diet. These eggs were delivered to the laboratory at the same time the individual eggs were placed in storage. The earlier study indicated that there is no relationship between pH and CO_2 content of egg white. The present study supports that conclusion, the value of the regression coefficient relating pH and carbon dioxide content of egg white for 45 pairs of observations on mixed egg white being $-.00122$. The value of the regression coefficient for the data on the stored eggs is $-.428$. A value of this magnitude does not indicate a significant correlation between pH and carbon dioxide content, since Fisher's criteria (l.c.) indicate that a value of this magnitude could be obtained more than once in ten trials.

The data given in Table VI would probably have more value if each egg stored had been paired with an egg laid by the same hen on the following day, the pH and carbon dioxide content of this second egg being determined within 24 hours after the egg was laid. A comparison of the values obtained for the paired eggs would give a better indication of changes that had occurred on storage.

Summary

Physical and chemical measurements of the eggs from groups of hens on five different rations, and physical measurements on sponge cakes made from eggs from the same source indicated that the diet these hens received did not affect the properties of the eggs or the quality of the cakes made from them.

No progressive change was found in the properties of the eggs or quality of the cakes over the period in the laying cycle of the hen or the seasons of the year studied.

There was no apparent relationship between the physical and chemical properties of the eggs studied and the quality values of the cakes.

There was a relationship between the specific volume, elasticity, and compressibility of the sponge cakes, and between the pH and specific gravity of the batter from which the cakes were baked.

Findings of an earlier study which indicated that there is no relationship between pH and CO_2 content of egg white were confirmed.

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DEVELOPING AND TESTING IMPROVED VARIETIES OF WHEAT

K. S. QUISENBERRY

Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture, Washington, D. C.

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Introduction

The production and distribution of improved wheat varieties in the United States are nothing new. Attempts to obtain better varieties were made in this country soon after wheat was first grown. It is possible to trace the history of Red May, a soft red winter, to about 1700. Flint and Purplestraw, also soft red winters grown in the southeastern states, were known in 1814 and 1822, respectively. Mediterranean, a variety grown in North Central Texas, was introduced prior to 1840; Zimmerman was selected in 1837, and Fultz in 1862. During the last 30 or 35 years the most progress has been made in wheat improvement due to the advancement of the knowledge of genetics, increased support of research work, and more interest on the part of farmers and seed growers.

Methods of Improving Wheat Varieties

There are three general methods used in the improvement of wheat varieties. The first is to introduce a variety from a foreign source in the hopes of getting something that will be better. Turkey wheat, introduced into Kansas from Russia, started the growing of hard red winter wheats in this country.

The second method of improvement is to select the desired type from another variety or commercial mixture. This method has been widely used by both private and public breeders with much success. Varieties such as Kanred, Blackhull, Cheyenne, and Denton are results of selection.

When both introduction and selection fail to produce the desired type it is necessary to resort to crossing. Many crosses have been made without first studying the characteristics of the parents and a few of these crosses have produced strains having some value. The more modern method is to determine the type of variety or improvement desired, carefully select parents that may be expected to give the hybrid these characteristics and then make the cross. In some

cases it may be necessary to make more than a single cross to obtain the desired combination, and at present some rather complex hybrids are being studied. Some of the outstanding results of crossing are Marquis, Ceres, and Thatcher spring wheats and Tenmarq winter wheat.

After an introduction, selection, or hybrid is obtained, a long testing period ranging from 5 to 15 years is needed before the new variety may be made available.

The State-Federal Plan of Cooperation

Throughout the United States, but especially in the Great Plains and in the area west of the Rocky Mountains, coordinated wheat improvement programs have recently been developed in which the State Agricultural Experiment Stations and the U. S. Department of Agriculture are cooperating. Three such programs in as many areas have been under way for 6 or 7 years each. These are located in the Pacific region, the hard red spring wheat region, and the hard red winter wheat region. This discussion will deal entirely with the hard red winter wheat program, but the plan of the others is much the same and all plans are more or less correlated with each other.

The program in the hard red winter wheat region includes 24 breeding or testing stations scattered from College Station and Amarillo, Texas, on the south to St. Paul, Minn., and Havre, Mont., on the north. At each of the breeding stations, usually one in each State, there is located a State or Federal man who devotes much or all of his time to wheat improvement. Here crosses and selections are made and necessary genetic and disease problems are studied. Promising varieties and strains are then grown at other stations where they may be studied under different climatic conditions. For the most part the stations, either breeding or testing, are located with reference to the important wheat production areas.

Pathologists are located at various places throughout the area to study diseases as well as to assist in breeding for disease resistance. Milling and baking laboratories are available at Bozeman, Mont., Fargo, N. Dak., St. Paul, Minn., Lincoln, Nebr., Manhattan, Kans., and Washington, D. C.

All of the improvement work in the area is coordinated into one large program. Within the State, problems of local nature and interest are studied, while from the Federal standpoint attention is given to problems of regional interest. Unnecessary duplication is discouraged, while necessary checking of problems in differing environments is encouraged.

At various stations it is possible to concentrate on the study of

specific problems, obtaining information usable for the entire area. For example, at College Station and Denton, Texas, leaf rust and stem rust are nearly always present, so these stations are ideal for testing the rust resistance of wheats. At Denton, Texas, lodging is often a factor due to heavy rainfall. Here it is possible to study factors causing lodging and to learn something of the ability of varieties to resist lodging. At Bozeman, Mont., it is relatively easy to obtain a good infection of bunt or stinking smut, while at Moccasin, Mont., or St. Paul, Minn., winter-killing is usually a factor, so at these stations extensive tests are carried for these characters. Artificial freezing equipment is available at St. Paul, Minn., Lincoln, Nebr., and Manhattan, Kans., making possible a study of cold resistance under controlled conditions. At any of these stations material from the entire area may be tested under conditions favorable to a maximum expression of the disease or condition, and the data are made available as soon as possible. Thus, it is felt that information can be obtained more rapidly, more completely, and more efficiently than if all the effort were individual.

How the Program Works

It is the aim of the program to produce wheats that will resist or escape as many hazards as possible and have good quality. It is desired to obtain varieties which may be expected to produce a crop under as wide a range of conditions as possible, thus making the crop more sure. In addition, it is the desire to give the farmer varieties that will give the greatest return per acre. At any given breeding station in the area new wheats are being developed with these objectives in mind.

During the early generations the strains are kept at one station at which they are tested as thoroughly as possible for resistance to diseases such as leaf and stem rust, loose and covered smut, foot rot and scab; resistance to insects, lodging, winter-killing, drought, heat, and shattering. After a strain has shown promise in these local tests and sufficient seed is available it is put into a uniform agronomic nursery where it will be grown and observed at about 12 stations under varying environments. Thus the strain may be subjected to more hazards or more severe conditions than would be possible at the single station. In this way several readings are obtained each year, in much the same way as the cereal chemist adds bromate to one bake, sugar to another, and varies the time of mix on others. It must be remembered that our laboratory stretches for hundreds of miles rather than a few feet. At the time a strain is put into the agronomic nursery it also enters the regional disease nurseries, and may be sent to other places for special tests.

The better strains from the agronomic nursery are selected for testing in field plots at the various stations and uniformly in the area, and after a careful study in field plots for a period of 3 to 5 or more years the best variety is ready for testing on farms. After a test as thorough as outlined, a great deal is known about a variety. The good points and the defects are understood. It is possible to predict fairly closely to what area the variety will be adapted, where it should do well, and where it will probably fail. The idea is to obtain this information and make it available at the time the variety is released.

Quality Tests

The problem in wheat would be more simple if the adapted variety, resistant to hazards, could be developed and we could stop there. However, this is not the case as the question of quality enters the picture. The new variety must not only be good agronomically but must have good quality. The quality must meet the requirements set by the trade for wheat grown in the area. The grain must have a good color and test weight, and must be hard, and the flour resulting be acceptable to the baker. It is assumed that most of the hard red winter wheat is used for bread baking.

At this point the fun starts. What measure shall we use in our quality tests? What is the goal? Some tell us to obtain high protein content, others say obtain varieties that will stand a certain type of mixing, others suggest the use of the bromate test, while others hold that reaction to bromate means little. In some places the wheat meal-fermentation time test is highly recommended as a measure of quality. To the poor agronomist this is confusing to say the least, especially since it is often hard to establish correlations between these tests. As long as trade demands differ it will probably be hard to get a wheat to suit all people, but since many varieties are grown and the seed is mixed in the bin, some of the difficulty may be eliminated.

In our work quality tests are started as soon as enough seed is available. Strains are first discarded on "looks"; later, protein determinations may be obtained. From the uniform agronomic nurseries and field plots, baking tests are made, often in more than one laboratory. In Washington, the strains are baked by the standard A. A. C. C. method by the use of the basic and several other bakes, the bakes varying with different years. Whenever possible identical material is baked in other laboratories, in order to obtain as much information as possible. In addition, several states are doing special work on varieties of local interest. If a strain is decidedly inferior for quality it is discarded from all tests. If it is outstanding, however, in its agronomic performance and possibly different only in quality, careful tests

are made to determine whether good bread can be made from the strain by methods available and usable in the average bake shop. If this is possible the variety would be considered acceptable.

The agronomist would welcome an acceptable quality test which could be applied to small amounts of seed, thus making possible the elimination of poor strains early in their history. The agronomist naturally leans on the cereal chemist for guidance on matters of quality and cooperates closely with him both in experimental and in commercial laboratories. This cooperation is very helpful and is greatly appreciated, for after all the variety must suit the buyer as well as the grower.

New Varieties

The farmer is constantly looking for better varieties and the plant breeder, both private and public, is working to produce them. The milling trade wants a constant supply of the same kind of wheat and often in the past has not been friendly to new varieties, especially when they are first introduced. Both good and bad varieties have been distributed; mistakes have been made by both private and public plant breeders. With the introduction of new varieties the trade often complains, loudly at first, continuing loud in some cases and in others softening as the variety becomes better known. During much of this complaining no material reduction in price is put into effect. A farmer will naturally grow the variety giving him the best yield so long as he gets the regular price, but if he is docked enough because his variety is not acceptable he will soon stop growing it. Is it a fair question to ask why there is not a stronger tendency to reduce the price on certain varieties in proportion to the complaints made against them?

Possibly some of the trouble is caused by the fact that the miller does not always know what he is getting until it is too late. If he could identify the variety, say 95 per cent of the time, he might not buy it. It is admitted that absolute identification of many of the varieties of hard red winter wheat is difficult even for the expert. Some can be identified by the grain, more by head and plant characters, while in other cases physiological or disease reactions are needed for certain identification. If the plant breeder could put identifying marks on the varieties it would be highly desirable. This is not entirely impossible but makes the job a little harder.

In Canada, some elevators follow the practice of keeping a small amount of each farmer's wheat and seeding it in a row near the elevator. At the next harvest a meeting is held and these rows are identified and examined for purity. Those farmers having undesirable varieties or mixtures are advised to get new seed or else market else-

where. Such a procedure might be useful in this country, especially in sections where many different varieties are grown, and should do much toward eliminating undesirable types.

Number of Varieties

The complaint is often heard that there are too many varieties of wheat being grown. There are too many varieties and, in spite of extension and other programs urging fewer varieties, the number is increasing. For example, in a varietal survey made in 1919, 8 varieties of hard red winter wheat were reported. In 1924, there were 17; in 1929, 28; and in 1934, 34 varieties reported on commercial acreages. In other words, new varieties are coming in more rapidly than old ones drop out. In Kansas, 7 varieties of wheat are recommended by the Crop Improvement Association. In the 1934 survey, 29 varieties were reported as being grown. In Nebraska, 4 varieties are recommended and 24 were reported as being grown. It is true that many of these varieties are grown on small acreages, but still they are in the state.

There are probably several remedies for such a situation. The first would be to forbid the production of new varieties. This would be foolish since varieties giving more assurance of a crop are needed. No doubt varieties with wider adaptation could be developed, although it is doubtful if another Turkey or Marquis will be produced. It is felt that with this cooperative program varieties of wider adaptation may be developed. It has been suggested that single variety communities be established. To accomplish this would require considerable legislation and much effort in administration. Probably the most practical way will be to reduce the number of new varieties coming out, first making very sure they are acceptable, and continuing the missionary work for fewer but better varieties. It must be remembered that this cooperative program has no control over varieties put out by private sources. The trade could be a big help if they would back their complaints by reduced prices on undesirable types.

It has been pointed out that there are many stations and men working on wheat improvement. If you are located in the Great Plains or on the Pacific Coast, you are not far from one of these stations. If you are interested in wheat varieties, visit these men and find out what is new and what may be coming out. Tell them the type of wheat you want, possibly invite them to come to see you now and then. In other words, get acquainted and let each be tolerant of the other's problems, and when a new variety is being distributed know about it early and not for the first time when Mr. Farmer pulls up to your elevator with a load of the new wheat. In this case anyone

would be suspicious, yet the variety no doubt has merit or else it would not have been released. If a more tolerant attitude could be developed, and it is developing in many sections, the trade would not think that plant breeders and agronomists are desirous of putting a different variety on each farm.

PRACTICAL SUGGESTIONS FOR THE PREPARATION OF STANDARD SOLUTIONS FOR PROTEIN TESTING [1]

W. O. WHITCOMB

Montana Agricultural Experiment Station, Bozeman, Montana

(Read at the Annual Meeting, June 1936)

The preparation of standard solutions in the protein testing laboratory is an important task and one which frequently requires considerable time. The suggestions given in this paper for the preparation of these solutions by weighing the materials have been found to be practical.

The method commonly employed of combining the chemical and the water in such a way as to make the solution slightly too strong and then adding water according to the results obtained by titrating against a standard, while productive of accurate solutions, involves considerable time. By the method herein described of weighing the chemical and the water, it has been found possible to make up solutions of NaOH and H_2SO_4 which, when titrated against a standard, have an accuracy of 1 part in 500.

This method of preparing solutions is best accomplished at a temperature of from 20° to 25° C., but reliable results can be obtained at slightly higher temperatures. Since the density of water at 20° C. is 0.998, it becomes necessary when weighing water to consider 998 grams as a liter.

Stock Chemicals

Two liters or more of the stock chemicals to be used in preparation of standard solutions should be placed in bottles capable of sealing airtight. A satisfactory method of preparing the NaOH stock solution is to place 1,500 g. of sticks in 2,000 cc. of water and to boil for 10 minutes. After the solution has cooled, it is placed in a stoppered bottle where it is allowed to settle for a few days. The clear solution is then transferred to the bottle in which it is to be stored. Concentrated chemically pure H_2SO_4 is used without special preparation, other than to seal it in a special bottle. Stock solutions of NaOH and

[1] Contribution from Montana State College, Agricultural Experiment Station. Paper No. 75, Journal Series.

H_2SO_4 have been found to remain constant for 5 years or more. It is possible, however, in some cases, that concentrated NaOH may react with glass, forming a silicate and thus suffer a reduction in strength.

Standardization of Chemicals

The first step in the preparation of standard solutions by weighing the chemicals and the water is to determine the amount of chemical required per unit weight of water. This procedure is illustrated by the following example:

Required: The amount of concentrated NaOH to add to 998 g. of water to make a 0.10 N solution.

A trial solution is prepared by adding 20.00 g. of concentrated NaOH to 1996 g. of water. This is divided into 2 equal parts. A 50 cc. portion of the first part is titrated against a 0.10 N pottassium acid phthalate or other standard solution, and it is found that it requires 50.00 cc. of the standard to neutralize 45.85 cc. of the NaOH. By the simple proportion, 45.85 : 4.15 : : 998 : x, it is found that it is necessary to add 90.3 g. of water to the second part to make it 0.10 N. This means that 9.17 g. of NaOH are required for each 998 g. of water. Knowing the amount of this particular lot of NaOH which is required to add to 998 g. of water to make a 0.10 N solution, the amount required for a 0.1253 N solution can readily be computed, and is found to be 11.49 g.

The amounts of the stock chemical required to make a 0.10 N or a 0.1253 N H_2SO_4 solution can be determined in the same manner as that described for the NaOH solutions.

The amounts of chemicals required for solutions of certain normalities are indicated in Table I. These amounts are only intended to be suggestive as it is necessary to determine the weight of each lot of chemicals used in preparing solutions. However, when these weights have once been established for a particular lot of chemicals, they can be used as long as the stock chemicals are well preserved.

TABLE I

WEIGHT OF CHEMICALS REQUIRED PER 100 G. OF WATER TO MAKE STANDARD SOLUTIONS, TOGETHER WITH CORRECTION FACTORS, AT 20° C.

Chemical [1]	Grams chemical per 998 g. water [2]		Change for each .10 g. of chemical per 988 g. water [3]	
	0.10 N	0.1253 N	0.10 N	0.1253 N
	Grams	*Grams*	*Cc.*	*Cc.*
NaOH	9.17	11.49	0.55	0.43
H_2SO_4	5.22	6.54	0.96	0.76

[1] NaOH, concentrated solution of 1,500 g. sticks in 2,000 cc. water. H_2SO_4, C. P., specific gravity 1.84.

[2] These weights apply only to the particular lots of chemicals used in this study.

[3] These changes based on 50 cc. portions of the solutions.

The correction factors, as given in Table I, are for the purpose of indicating the degree of accuracy expected in weighing out the various chemicals. They can also be used to advantage in modifying solutions which are found to be weak. For example, if a solution of NaOH, when titrated with 50 cc. of 0.10 N standard, is found to require 51.10 cc., it will require 2 × .55 g. of the stock NaOH to bring the solution under consideration up to 0.10 N.

Preparation of Solutions in Large Quantities

This method of combining chemicals and water by weight according to definite proportions worked out for stock chemicals is especially well adapted to the preparation of standard solutions in 40 liter lots or more. The stock chemicals are readily weighed by placing a beaker, which has been rinsed with distilled water, dried in an oven and cooled, on a balance and adding the chemical with a pipette. Weighing to the nearest decigram is sufficient. The water is weighed in bottles of approximately 1 or 2 liters capacity, and read to the nearest gram. If the conditions are not such that the water can be weighed, it can be measured with volumetric flask.

TABLE II

WEIGHT OF 100 CC. PORTIONS OF STANDARD SOLUTIONS OF GIVEN NORMALITIES AT 20° C.[1]

Solution	0.10 N	0.1253 N
	Grams	*Grams*
NaOH	100.46	100.57
H_2SO_4	100.35	100.44

[1] These weights are useful in comparing solutions with standards by the gravimetric method.

Gravimetric Method of Comparing Solutions with Standards

It has been found satisfactory to check solutions against standards, by the gravimetric method, after the normality has been established by the volumetric method. The weights of the solutions as given in Table II are used for this purpose. A NaOH solution can readily be checked with potassium acid phthalate by placing 100.46 g. of the NaOH solution in a flask and adding 2.0414 g. of the phthalate. When the phthalate is dissolved, add phenol phthalein indicator and note the color.

REPORT OF THE 1934–1935 COMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

H. G. WALTER, *Chairman*

Igleheart Brothers, Incorporated, Evansville, Indiana

(Read at the Annual Meeting, June 1935)

At a meeting of the Committee in Chicago before collaborative work was under way, two minor changes were made in the scoring system (Walter, 1933 [1]).

First, flavor, having a value of 20 points, was more closely defined as follows:

	Perfect	Faulty
Flavor:		
Flour quality (10 points)	Normal	Wheaty, starchy, lacking
Eating quality (10 points)	Tender, friable	Doughy, dry and mealy, tough

Second, a penalty system to be applied to the total score was arranged so that a biscuit whose measured score might be good while at the same time being inferior due to some unusual characteristic of the flour (such as a strong, absorbed or developed odor, or some objectionable peculiarity of the dough) could be suitably marked down. The following example illustrates how a flour with an otherwise fair score may be penalized and all doubt removed as to its unsatisfactory characters:

Total score	75
Penalty	50
Final score	25

Reasons: Slightly musty, or rancid, or garlicky, *etc.*

The mixing and handling procedures remained the same as were used last year (Walter, 1935 [2]), but the Committee decided to increase the dough batch to have an extra biscuit available for flavor scoring while hot. The recipes are compared below:

[1] Walter, H. G., 1933, Tests for biscuit and self-rising flours. Cereal Chem. **10**: 635–641.
[2] Walter, H. G., 1935, Report of the 1933–34 Committee on Methods of Testing Self-Rising Flours. Cereal Chem. **12**: 161–164.

	1934 Recipe	1935 Recipe	Percent
Flour (15% moisture basis)	200 g.	227.7 g.	94.90
Sodium bicarbonate	3 "	3.4 "	1.42
Monocalcium phosphate	3.75 "	4.3 "	1.78
Salt	4 "	4.6 "	1.90
	210.75 g.	240.0 g.	100.00
Self-rising flour (basis 15% moisture in the plain flour)			240 g.
Hydrogenated shortening			30 g.

Collaborative Baking Work

Samples of self-rising flour from one source were distributed to the collaborators for the purpose of determining the extent of agreement with respect to absorption. It was first decided to determine whether the absorption of the sample as indicated by a farinograph might be used as the optimum absorption in the biscuit baking test. The farinograph indicated a figure of approximately 53% absorption, which was decidedly low as subsequent work indicated an absorption of 65.8% under actual baking conditions.

Three collaborators reported as the optimum absorption figure 65.8%, while the fourth preferred 63.2%, with 65.8% as second choice. The optimum absorption was selected by baking a series of biscuits varying the absorption in steps of 2½%.

Since agreement on the absorption figure of one common lot of flour was considered satisfactory, further collaborative bakings were carried out on three soft wheat flours of different qualities to determine how the flours would be classified by means of the biscuit baking test. The analyses of the flours used follow:

Flour	No. 1	No. 2	No. 3
Ash	.420	.480	.364
Protein	9.30	9.62	9.02
Moisture	12.83	12.70	12.33
pH	5.35	5.30	5.20

Each of the collaborators classified the flours in the same order—Nos. 3, 1, and 2, as shown by the following total scores:

Flour	No. 3	No. 1	No. 2
Collaborator A	94.6	92.0	88.2
" B	95.0	91.4	88.8
" C	97.6	94.1	85.4
" D	94.6	91.2	85.9

Discussion

The laboratories with gas ovens showed higher oven losses than did those with electric ovens.

Higher oven losses were accompanied by greater specific volumes.

Although the collaborators reached agreement with respect to the comparative baking qualities of the three flours, the individual total scores differed to some extent. This was attributed to the fact that score items have not been suitably standardized. In view of the comparisons possible with adequate flour standards, which help to eliminate the necessity for closely standardizing each of the scoring items, the committee favors the establishment of some method for selecting flour standards. For the consideration of a future committee, we suggest that work be done to establish a standard deviation of results using the baking test on a single sample of flour; after that an investigation would be advisable to determine whether soft wheat flours conforming to certain analytical standards would be sufficiently constant in baking quality to warrant the setting of standards on an analysis basis. Confining the test to soft red winter wheat flours for the present, the more influential items would appear to be ash, protein, pH, and color. The first two offer no problem to the cereal laboratory, while the measurement of color promises soon to be within reach. Through the courtesy of the Wallace and Tiernan Company, the committee has been carrying out some preliminary tests on adapting the N–A Disc Colorimeter to the standardization of biscuit flour colors.

Acknowledgment

The chairman wishes to acknowledge the constant assistance and cooperation of R. A. Barackman, F. A. Collatz, and H. V. Moss, who constituted the committee.

REPORT OF THE 1935–36 COMMITTEE ON EXPERIMENTAL BAKING TEST

C. O. SWANSON, *Chairman*

Kansas Agricultural Experiment Station, Manhattan, Kansas

(Read at the Annual Meeting, June 1936)

This year's committee on Experimental Baking Test comprised all those who made reports last year at Denver on the work of the committee, plus three additional members. In arranging for this year's work, the committee gave consideration to what each member had previously done as well as his desires. There were two objectives in mind—(1) continuation of the previous work of the committee so as to conclude work already under way, and (2) the incorporation of some new studies. This arrangement of the committee work will be made clear as the individual reports are given.

What Should be the Baking Procedure?

Collaborative baking tests by a number of cereal chemists have frequently been used by the laboratory at Kansas State College for the testing and evaluation of flours from important wheat varieties. Each participating chemist tests the flours in his own way and evaluates them in the order of his preference. By this method directly opposite conclusions are often reached, that is, a flour put in the last place by one chemist may be placed in the first place by another. Commenting on this situation, a prominent mill executive made this statement: "The cereal chemists are either inefficient or they lack proper apparatus or their shop conditions vary widely." I do not believe any of the statements just quoted are the reasons for the disagreements. Answering another critic of cereal chemists, I made this statement: "If half a dozen of the best physics technicians were asked to measure the width of a room with a rubber tape, there would not be any agreement in the report." The situation in regard to disagreements on the results of the baking test has suggested several thoughts which I want to express as my part of the report.

The main reason why conflicting results are often obtained in the baking test is that the behavior of flour in the process of bread making and the quality of the bread obtained depend on two sets of factors—(1) the inherent characteristics of the flour, and (2) the methods and

conditions of making the tests. If the general scheme of the baking procedure is adapted to the characteristics of the flour the results in the bread will be satisfactory, and if they are not so adapted the results will be unsatisfactory; that is, final results depend both on methods of procedure and inherent quality characteristics.

An Adequate Baking Test Must be Comprehensive

This situation indicates that the baking test has two objectives: (1) to discover the inherent characteristics of the flour, and, (2) to find the method of baking procedure which will get the best results from these inherent characteristics. This implies that the baking test cannot in the very nature of the case be made a single or simple test, but it must cover the different variables which may be introduced. At a meeting in Lincoln, Nebraska, last year, Dr. Blish presented a large number of loaves baked from a single wheat variety. This was done to bring out the inherent baking characteristics of the flour from that wheat and also to find the procedure best adapted to get the best results. Rowland Clark wants to bake at least 12 loaves for each variable introduced. This would involve the baking of 36 loaves if the variables included were absorption, mixing time, and fermentation time. Such a program would make for a heavy day's work even with the help of assistants. If more variables were introduced, another day of testing would be required. This situation means that the number of flour samples which may be tested comprehensively is rather limited, and that the single, simple baking test is too limited in scope as a measure for evaluating an unknown flour. In this situation, what can be done?

Interpretation of the Specific Baking Tests

It would seem that the direction in which we should be going is to make specific tests on the wheat or flour and then learn how to interpret the results in terms of their behavior in the bakeshop. Some people object to making interpretations, but has not much of the confusion come from an endeavor to find methods whose results do not need interpretation? Most of the specific tests needed are already known. Some of them may require further development and refinement, but that road is easy to travel. The main thought and effort should be to find an adequate basis for making interpretations, that is, on the basis of the results of these specific tests make forecasts of how the flour should be handled in order to get satisfactory results. This may be contrary to prevailing ideas, but is not this the fundamental principle followed in some other professions such as medicine and engineering? The physician takes the pulse, reads the heart beat, listens to the respiration,

makes the blood count or other determinations. Some of the laboratory tests are made by technicians, but the physician's success or failure depends on his ability to make the diagnosis or the interpretation. Similarly, the engineer must know from specific tests the strength as well as other characteristics of each kind of material which goes into a structure. Thus, when he builds a bridge he knows before the excavation starts for the foundation, that the bridge will carry not only itself but the intended loads.

Some Specific Tests

Our profession is relatively young and we have not arrived where the physician and the engineer are now, but even they are reaching out for further conquests and perfection. It is, however, of paramount importance for us to determine the direction in which we should go. To illustrate, a few specific tests will be mentioned.

Protein content.—The protein is probably of first importance in bread wheats, both quantity and quality. For best results in bread making it is necessary to have a certain amount of protein in the flour, say from 10.30% to about 12.0%. We know that very low protein wheats will not make satisfactory bread flour, and also that very high protein wheats are not necessary. We also know that there are fundamental differences in protein quality, at least the colloidal protein complex is such that several flours of exactly the same protein content behave very differently. There are available several specific tests which reveal these differences in protein quality, and we need to learn their meaning so as to be able to predict how the flour will give best results in the bake shop.

Flour yield.—We have done comparatively little on developing a method which shall satisfactorily measure the milling quality as far as that is related to the mechanical separation. The test weight is a rough measure of the flour yield, but we know that it is far from adequate. We need a much more accurate measure. May I suggest that the development of a precision mill is one of the promising possibilities.

Gassing factors.—The determination of diastatic activity and the closely related gas-production capacity can be done by several satisfactory methods. There are also satisfactory methods for measuring gas retention in dough. We know that adequate gas production is one of the first essentials needed to produce the loaf volumes and the light crumb textures desired, but without the needed gas retention, abundant gas production is of no avail. Defects due to insufficient gas production are more easily remedied than defects due to inadequate gas retention which is closely dependent on the inherent colloidal properties of the proteins. From the results of the diastatic activity determination or gas

production as well as the measure of gas retention, we should learn whether we are going to have the right balance between gas production and gas retention in dough at the time needed so as to have the desired volume and crumb texture.

Mechanical tests.—Modern factory bread making is mostly a machine process. For this reason the behavior of dough under mechanical stress is one of its most important characteristics. There are various methods of measuring this behavior and need not be mentioned here. Our European friends seem to have advanced further with the mechanical methods than we have. It was but natural that chemical tests should have taken the lead in the past because most of the workers in wheat and flour testing were trained as chemists. As we advance we are learning that physical tests have their place along with the chemical. We have, however, a long way to go in the interpretation of these tests, but progress is being made. We can now predict quite accurately the amount of mechanical mixing needed as well as the danger of over-mixing. There are besides several dough handling properties which may be determined. Since fermentation involves a certain amount of mechanical stress, these measures of resistance to stretching also offer information on the behavior of the dough during fermentation.

Interpretations Needed

The picture I want to leave with you is this: The baking qualities of a flour are determined by a number of characteristics more or less interrelated. We have made satisfactory progress in determining a goodly number of these characteristics. While there is plenty of technique yet to be learned, the most important task before us is to learn how to interpret these definite determinations not only in terms of potential baking value based on inherent properties, but also in terms of methods adapted to bring out the best results possible because of these inherent properties. In proportion, as we learn to do this, we shall gain more assurance that our work rests on a better foundation.

The Baking Test Will Remain

This does not mean the passing of the baking test. While we are learning the interpretation of characteristics which can be definitely determined, we need to continue the baking tests. However, in the further development of the baking test we should tie in as closely as possible with the actual practices in the bakeshop. Referring to a published story by our president, "We need to learn the bakers' language."

THE PRESENT STATUS OF THE "STANDARD" A.A.C.C. BAKING TEST [1]

M. J. BLISH

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Read at the Annual Meeting, June 1936)

My small part in the activities of the present Experimental Baking Test Committee consists of an effort to estimate, in a general way, the progress that the Association has made thus far in its endeavor to establish a "standard" laboratory baking test. I will briefly present my conclusions for two reasons: (1) I have been closely associated with the project for the past 10 years as a member of each of the Committees, and (2) I believe that we have definitely reached a situation that calls for drastic action of one kind or another.

Year after year our Baking Test Committees submit their respective reports of work that represents, in the aggregate, an enormous amount of time, thought, expense, and conscientious effort. Although we designate the Committee as the "Committee on *Standardization* of the Experimental Baking Test," much of the work by it has dealt with matters of interpretation rather than standardization. Very seldom do we pause to give serious consideration to the progress, if any, that is being made in terms of the definite objective that we are supposedly trying to reach.

I am in perfect agreement with A. G. Simpson, the British chemist, who insists that our ultimate responsibility and goal is to be able to specify flour baking properties in standardized, simple terms, preferably numerical values. If the baking test is actually and admittedly the only trustworthy means for the identification and measurement of these properties, then the obviously simple fact is that the establishment of a *standard* baking test procedure offers the only means for accomplishment of this purpose. I maintain that we are no nearer our ultimate objective than we were 5 or 6 years ago, by which is meant that our so-called "standard" test is no nearer "standard" now than it was then.

Most of us now recognize and agree that there is no *single* baking test that will serve to measure all of the important flour properties, and that these must be registered in terms of "response" to specified treatments. Hence, we have the basic and supplementary procedures,

[1] Subcommittee report, 1935–36 Committee on Standardization of Laboratory Baking.

the basic procedure being the reference point from which *response* to the supplementary treatment is to be measured. In officially adopting a fixed and controlled basic method, allowing but one variable, we applied strictly scientific principles for the first time, but a method can be scientific without being *standard*.

In taking stock of the situation, I submit that we should first of all consider carefully the tremendous difficulties that are involved in establishing a standard basic procedure that is worthy of the name "standard." Our investigations have at least made clear that the successful fulfillment of this endeavor means the control and *mechanization* of all operations. It means a specified formula, a specified mixer, a specified mechanism for punching and molding, a specified pan, and a specified oven. Nothing less than the strictest specifications for all of these features can accomplish our purpose.

The seriousness of the problem becomes more apparent when we realize that the situation demands, for instance, the adoption of *one* oven, made by *one* manufacturer. The same applies to the mixer, the mechanical molder, and the pans. Many will say that it simply cannot be done, and perhaps they are right. Theoretically, at least, it is possible to decide upon one piece of equipment, made to specification by one manufacturer. But it seems that we now encounter the more difficult element of human nature.

This is well illustrated in the case of baking pans. Different workers prefer different pans, and each one declares that he simply will not give up the type of pan to which he has become accustomed. I have repeatedly heard such declarations made by members of the Baking Committee themselves. As a compromise, the latest official specifications permit both low- and tall-form pans, which is, of course, absurd from the standpoint of standardization. This attitude, alone, will defeat any chances for standardization, and will make further efforts toward standardization a waste of time.

As I see it, we are clearly faced with an issue that involves two and only two alternatives. One is that an officially appointed committee shall, after due deliberation and study, issue an ultimatum specifying arbitrarily each piece of mechanical equipment, as well as the manufacturer of the equipment. There is no other way. This will necessitate the large scale purchases of new and standard equipment. It will mean the relinquishing of individual customs and preferences for the good of the common cause. It will mean, in many cases, adjusting one's self to interpreting a different type of loaf than the customary one. It will cause the irritation and, in some cases, the violent hostility of certain equipment manufacturers. The inevitable consequence of all this will be that, at first, most technicians

will refuse to even consider using the test, and even if they eventually decide to do so, a large number of executives will refuse to spend the necessary funds for new and standard equipment. Any general adoption and use of the standard method would be at best slow and gradual, but we would at least have a "standard" method, or the nearest possible approach to it. Do we want the standard method badly enough to be willing to subject ourselves to this painful process?

The other alternative is to drop all pretense of calling the present method a "standard" test, and simply recognize it as an "official" or an "approved" type of procedure. This is much the easier course to follow, but in adopting it, we admit defeat in our efforts to reach what I have maintained should be the ultimate objective of cereal chemistry as an applied science, namely, to be able to specify, in simple standard terms, those properties of our principal raw material, flour, that admittedly can be identified and measured only by test baking.

There are, to be sure, a few who feel that we are rapidly approaching that fortunate state of affairs in which the baking test will be discarded in favor of physical measurements. If that is indeed true, which I personally doubt, then it is doubly apparent that any further heroic efforts to establish a *standard* baking test, in the true sense of the term, should be abandoned.

We owe it to ourselves to designate clearly our future policy with reference to the experimental baking test. Is it to be *standard* or merely *official?* It is unfair to future committees to ask them to continue their labors under false pretenses.

REPORT OF THE 1935–36 COMMITTEE ON TESTING PIE FLOURS

C. B. KRESS, *Chairman*

Sperry Flour Company, San Francisco, California

(Read at the Annual Meeting, June 1936)

The 1935–36 Committee on Testing Pie Flours was made up of the following members: C. B. Kress, *Chairman*, W. K. Marshall, and P. O. Hastings.

Two members of the committee are engaged in the manufacture of soft wheat flours, and one member of the committee is engaged in the pie manufacturing business.

The purpose of this past year's collaborative work was to further our knowledge of the testing of pie flours and to arrive at a uniform and correct procedure for the evaluation of flours for pie making purposes.

Three samples of flour were studied—a soft wheat flour containing 7.5% protein, a hard winter wheat flour containing 11.3% protein, and a medium strong soft wheat flour containing 10.0% protein, which latter type of flour is commonly used as a cracker sponge flour.

The procedure followed in testing each flour was essentially that described in the 1934–35 report (Cereal Chem. **13**: 104–112) and embodied in addition to the chemical tests, viscosity tests, shortening requirements, and baking tests of pie shell making quality, as well as tests to determine the utility of the flours for filled pie purposes. Bread baking tests were eliminated from this year's study.

Data relative to the viscosity tests are given in Table I, along with the data relative to the chemical analysis of the flours. Curves illustrating the viscosity data are shown as Figure 1. The results of the pie shell baking tests as well as the filled pie baking tests are recorded in Table II. In Table III are to be found the results of the study relative to the shortening requirements of the flours.

Viscosity Tests

Flour No. 1 was the best pie flour of the three studied. This flour had a very low viscosity. Further, its viscosity increased very slowly with additions of lactic acid.

Flour No. 2 had the highest viscosity. The contour of the curve in Figure 1 shows a very rapid increase in viscosity with the first and second additions of lactic acid. Plainly its viscosity is that of a fairly strong bread flour.

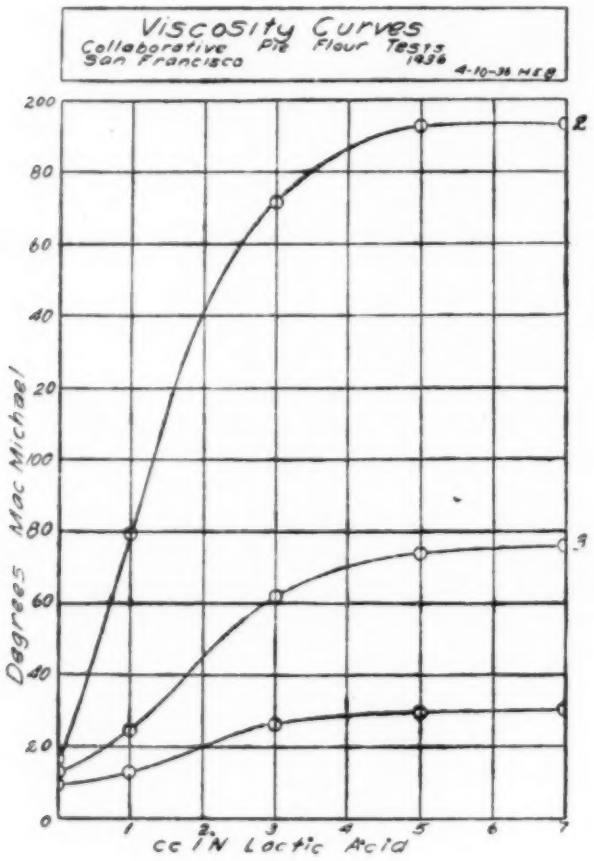

Fig. 1.

Pie tests with this flour indicate that it is not well suited to pie making purposes; hence, it is concluded that high viscosity is not favorable in a pie flour.

Flour No. 3 was of medium viscosity. The pie flour tests indicate, however, that it was just a little too strong. The collaborative tests of last year showed that the best pie flours had a maximum viscosity of 25 to 40° MacMichael. Flour No. 3 had a maximum viscosity of about 75° MacMichael.

TABLE I

CHEMICAL ANALYSIS OF THE FLOURS STUDIED

Collaborator	Ash	Protein	Clearness	Color shade	Absorption	Viscosity
Flour No. 1	%	%			%	° *MacM.*
Marshall	0.42	7.7	Clear	White	—	—
Hastings	.42	7.6	Clear	White	—	7–13–30–34–34
Kress	.42	7.6	Clear	Sl. cream	58	9–13–27–30–31
Flour No. 2						
Marshall	.39	11.3	Clear	Sl. grey	—	—
Hastings	.40	11.2	Clear	Brownish cast	—	16–89–187–205–208
Kress	.39	11.3	Clear	Sl. grey	64	13–79–172–193–193
Flour No. 3						
Marshall	.42	10.0	Clear	Yellow	—	—
Hastings	.43	10.0	Clear	Yellow	—	11–21–68–76–76
Kress	.42	10.0	Clear	Yellow	57	14–24–62–74–76

TABLE II

RESULTS OF PIE BAKING TESTS

	Collaborators		
	Marshall	Hastings	Kress
Flour No. 1			
Pie Shells			
Degree of baking	Good	Good	Good
Shrinkage	Very little	None	Very little
Color	Brown	Light	Dull brown
Flakiness	Good	Good	Very good
Tenderness	Good	Good	Very good
Crispness	Good	Good	Very good
Filled Pies			
Synthetic filling, sogginess	Top ¼ Bottom ¼	Crisp, dry Best of 3 samples	½ soaked still ½ soaked tender
Peach filling, sogginess	Top ¼ Bottom ¼	Same as above Same as above	½ soaked still ⅔ soaked tender
Flour No. 2			
Pie Shells			
Degree of baking	Good	Sl. soft	Good
Shrinkage	Little	Considerable	Considerable
Color	Golden brown	Sl. brown	Rich brown
Flakiness	Good	Compact	Fair
Tenderness	Tender	Tough	Tough
Crispness	Good	Soft	Soft and tough
Filled Pies			
Synthetic filling, sogginess	Top ¼ Bottom ¼	Soft, tough Very soggy, poorest of the 3 samples	½ soaked ⅔ soaked tough
Peach filling, sogginess	Top ¼ Bottom ¼	Same as above Same as above	½ soaked ⅔ soaked tough

TABLE II—*Continued*

	Collaborators		
	Marshall	Hastings	Kress
Flour No. 3			
Pie Shells			
Degree of baking	Good	Fairly good	Good
Shrinkage	Little	Slight	None
Color	Light brown	Med. brown	Rich brown
Flakiness	Compact	Fairly good	Very good
Tenderness	Sl. tough	Fairly good	Fair
Crispness	Good	Fairly good	Fair
Filled Pies			
Synthetic filling, no sogginess	Top ¼ Bottom ¼	Fairly dry Med. sogginess—next to No. 1	½ soaked ½ soaked } tender
Peach filling, sogginess	Top ¼ Bottom ¼	Same as above Same as above	½ soaked ½ soaked } tender

Remarks relative to the various flours:

Flour No. 1
- Hastings—least soaking; dryest top; crust most flaky; no shrinkage; most tender.
- Kress—most flakey; least soaking; very little shrinkage; most tender.
- Marshall—best pie flour.

Flour No. 2
- Hastings—compact; toughest; most soggy bottom crust; most shrinkage.
- Kress—compact; toughest; very soggy; most shrinkage.
- Marshall—poorest pie flour of the three.

Flour No. 3
- Hastings—next to No. 1 in tenderness, flakiness, and shrinkage.
- Kress—next to No. 1 in tenderness, flakiness, and shrinkage. No. 1 is the best pie flour.
- Marshall—No. 1 is best—and No. 2 is second best.

The results of the viscosity tests indicate that this test is one of the best we can apply to denote pie flour quality.

General Conclusions

The pie-dough formula and procedure recommended in the 1934–35 report of the Committee on Testing Pie Flours are well suited for testing pie flours. The formula is average as to shortening, and the procedure follows good commercial practice.

The committee believes that either the synthetic filling or the peach filling can be used. The synthetic filling has the advantage that it can be made up in quantity and kept ready for use at any time. The peach filling has the advantage that it makes a good eatable pie, and seems to make a more natural top crust.

The bread baking test gives some information, but is not of particular value alone to indicate the suitability of a flour for pie crust use.

The viscosity test gives the best information of any single test as to the adaptability of a flour to pie making purposes.

TABLE III

SHORTENING REQUIREMENT OF FLOURS

Collaborator	Percentage of shortening		
	50	60	70
Flour No. 1			
Hastings	Flaky, tender, crisp	Flaky, crisp, more tender	Mealy, very crisp, very tender
Kress	Tough, hard, compact	Short, flaky	Very short; very flaky
Flour No. 2			
Hastings	Compact, soft, tough	Sl. crisp; med. tough	Tender, med. crisp
Kress	Very hard and tough	Hard, compact, tough	Short, still too solid and compact
Flour No. 3			
Hastings	Med. flaky; med. crisp; med. tender	Flaky, tender; med. crisp	Flaky, crisp, very tender
Kress	Flaky, fairly short. Not quite enough shortening	Short, flaky. Tender, okay	Very short; very tender. Very short; not quite as tender as No. 1

Remarks:

Hastings

70% of shortening is too much for flour No. 1; satisfactory for flour No. 3 and not enough for flour No. 2.

Kress

Flour No. 1 is a good pie flour. The crust will soak, but still be tender.

Flour No. 2 would be hard to use for a pie flour; too tough and would require a lot of shortening.

Flour No. 3 is a good pie flour, but will require about 10% more shortening than flour No. 1. Pies from flour No. 3 could be handled more without damage than pies made from flour No. 1.

To arrive at a complete understanding of the quality of a pie flour it is necessary to make the following tests: moisture, ash, protein, color, viscosity, absorption, pie crust, and filled pie baking, as well as to determine the shortening requirements of the flour to make a good pie shell.

Recommendations

The committee recommends:

That the pie testing collaborative studies be continued.

That our procedure be adopted as the official method of the A. A. C. C. for testing pie flours.

REPORT OF THE 1935–36 COMMITTEE ON CAKE BAKING TESTS AND SELF-RISING FLOURS

L. D. WHITING, *Chairman*

Ballard & Ballard Company, Inc., Louisville, Kentucky

(Read at the Annual Meeting, June 1936)

The personnel of this committee, formed by the merger of the Cake Flour Committee and the Self-Rising Flour Committee, is as follows: J. W. Montzheimer, H. R. Fisher, J. R. Davies, W. E. Stokes, R. A. Barackman, H. W. Moss, and L. D. Whiting, Chairman.

In addition to these members, three collaborators assisted in the work of the committee as follows: M. D. Mize, Laura K. Track, and L. Armstrong.

The work of the committee falls into two divisions—the Cake Division and the Self-Rising Flour Division.

Cake Baking Tests

The recommendations of the 1934–35 Committee on Cake Baking Tests (Cereal Chem. **12**: 424) indicated (*a*) the desirability of a study leading to the preparation of type photographs to be used as an aid in classifying external and internal characteristics of test cakes, (*b*) the preparation of a chart which will show the necessary adjustment of the quantity of soda and cream of tartar for different atmospheric pressures, and (*c*) suggested the alternative use of fresh or frozen egg albumin in the official formula.

The studies of the Cake Division of the committee have been directed as far as possible along the lines suggested by the previous committees.

J. R. Davies has continued the work on photography which was started last year.

W. E. Stokes has worked extensively independently as well as collaboratively throughout the year on egg albumin.

H. R. Fisher was assigned the problem of investigating the influence of different types of shortening on the experimental cake baking test.

The collaborative work of the Cake Committee as a whole was centered on the problem of egg albumin, and the effect on relative scores when using various types of albumin.

Three flours were used in the collaborative baking trials. Their approximate analyses are as follows:

The collaborators were asked to bake these flours according to the basic cake baking method of the A. A. C. C. (Cereal Chem. **10**: 627),

Flour	A	B	C
Moisture, %	13.0	11.0	12.1
Protein,[1] %	8.0	8.0	9.3
Ash,[1] %	.34	.33	.409
pH	5.0	5.25	5.6

[1] 13.5% moisture basis.

and score the cakes according to the score card (Cereal Chem. 8: 253). Dried albumin was used in one set of tests, frozen albumin in another set, and fresh albumin from shell eggs in the third set.

All collaborators used the same dried albumin. This albumin only approximated the A. A. C. C. specifications. The fresh albumin and the frozen albumin were obtained locally by each collaborator.

The results of the collaborative baking tests are shown in Tables I to IV, inclusive. Table I is a summary of the collaborators' total scores

TABLE I

SUMMARY OF COLLABORATORS' SCORES USING FLOURS A, B, AND C, WITH DRIED, FRESH, AND FROZEN ALBUMIN

Type of albumin	Dry			Fresh			Frozen		
Flour	A	B	C	A	B	C	A	B	C
Collaborators	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*
A	92.0	90.0	72.0	95.0	90.0	73.0	91.0	88.0	73.0
B	67.2	74.4	74.15	82.5	89.0	84.85	81.4	87.25	84.45
C	86.0	93.3	85.0	88.7	96.3	90.5	88.3	94.8	88.2
D	71.5	77.0	69.0	83.0	83.5	69.0	79.0	85.5	63.5
E	86.0	86.7	81.2	86.2	87.7	83.2	86.0	86.2	84.2
Average score	80.5	84.28	76.27	87.08	89.3	80.11	85.14	88.35	78.67
Average score ratings of flours	2	1	3	2	1	3	2	1	3

when using flours A, B, and C. In Table II the volume scores obtained by each collaborator using flours A, B, and C are recorded. Table III lists the cake scores after deducting the volume figures given in Table II. Table IV records the texture, grain, and color values reported by each collaborator when using flours A, B, and C.

Comments of the collaborators.

COLLABORATOR A. This collaborator believes that the tentative method offered by the A. A. C. C. is not as valuable for testing cake flour as is a formula which is somewhat richer. It is his experience with this method that flours with low pH treated heavily with chlorine gave the best results in the test but did not work as satisfactorily in the bake-shop. It is his experience that a richer formula

gives results which more closely parallel bake-shop results and produces better cakes at a pH of 5.2. A favors the use of fresh or frozen albumin in the formula.

COLLABORATOR B. It is evident the collaborators have widely different conceptions as to the meaning of the various terms on the score card. This is brought out by comparing the scores of all collaborators when using dried albumin. This situation indicates that photographs should be used as a standard.

Three of the five collaborators did not experience much difference when dried, fresh, or frozen albumin, was used. Two collaborators found that fresh or frozen albumin is far superior to the dried albumin. In no instance did the fresh albumin give poorer results than the dried albumin.

It is interesting to note that the use of different albumins did not change the relative merits of the three flours. Collaborator B favors the use of fresh albumin officially, as the results in differentiating the flours were equal regardless of which

TABLE II

VOLUME SCORES OBTAINED BY THE COLLABORATORS USING FLOURS A, B, AND C, WITH DRIED, FRESH, AND FROZEN ALBUMIN

Type of albumin	Dry			Fresh			Frozen		
Flour	A	B	C	A	B	C	A	B	C
Collaborators	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*
A	14.0	13.0	12.0	15.0	13.0	13.0	13.0	13.0	13.0
B	12.2	12.9	13.65	13.5	14.0	14.85	13.9	14.25	14.95
C	11.0	12.5	12.0	12.0	13.5	14.0	12.0	13.5	14.0
D	11.5	16.0	16.5	16.0	17.0	16.5	13.0	15.5	15.0
E	12.0	12.5	12.0	12.5	12.75	12.75	12.5	12.75	12.5
Average volume score	12.14	13.38	13.23	13.80	14.05	14.22	12.88	13.80	13.89
Average volume rating of flours	2	1	3	3	2	1	3	2	1

TABLE III

COLLABORATORS' SCORES USING FLOURS A, B, AND C AFTER DEDUCTING VOLUME SCORES

Type of albumin	Dry			Fresh			Frozen		
Flour	A	B	C	A	B	C	A	B	C
Collaborators	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*
A	78.0	77.0	60.0	80.0	77.0	60.0	78.0	75.0	60.0
B	55.0	61.5	60.5	69.0	75.0	70.0	67.5	73.0	69.5
C	75.0	80.8	73.0	76.7	82.8	76.5	76.3	81.3	74.2
D	60.0	61.0	52.5	67.0	66.5	52.5	66.0	70.0	48.5
E	74.0	74.2	69.2	73.7	75.0	70.5	73.5	73.5	71.7
Average score	68.4	70.9	63.04	73.28	75.26	65.9	72.26	74.56	64.78
Average rating of flours	2	1	3	2	1	3	2	1	3

TABLE IV

SUMMARY OF TEXTURE, GRAIN, AND COLOR SCORES OBTAINED BY THE COLLABORATORS USING FLOURS A, B, AND C, WITH DRIED, FRESH, AND FROZEN ALBUMIN

Type of albumin	Dry									Fresh									Frozen								
	Texture score			Grain score			Color score			Texture score			Grain score			Color score			Texture score			Grain score			Color score		
Flours	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Collaborators A	28.0	26.0	20.0	23.0	23.0	18.0	13.0	14.0	10.0	29.0	26.0	20.0	24.0	23.0	16.0	13.0	14.0	10.0	26.0	26.0	20.0	25.0	23.0	16.0	13.0	12.0	10.0
B	22.5	23.5	24.0	11.5	13.5	16.5	11.0	12.5	8.0	26.0	26.0	28.0	21.0	22.0	23.0	12.0	13.0	8.0	26.0	25.0	27.0	20.0	22.0	23.0	11.0	12.0	8.0
C	24.5	29.8	25.0	24.0	24.5	22.0	14.0	13.5	12.5	25.0	30.0	26.0	24.5	24.5	22.7	14.2	14.0	13.0	25.0	29.5	26.0	24.2	24.0	22.5	14.1	13.5	10.9
D	22.0	24.0	20.0	14.0	12.0	13.0	11.0	13.5	8.0	25.0	25.0	22.0	15.0	17.0	13.0	13.0	11.0	5.0	24.5	26.0	20.0	15.0	17.0	12.5	13.0	13.5	5.0
E	26.25	26.5	25.25	22.25	22.25	20.5	13.0	13.0	11.75	26.25	26.5	25.0	21.75	22.25	21.0	13.0	13.5	12.0	26.0	26.0	25.0	22.25	22.0	21.5	13.0	12.75	12.25
Average	24.65	25.95	22.85	18.95	19.05	18.0	12.4	13.3	10.05	26.25	26.7	24.2	21.25	21.75	19.14	13.04	13.1	9.6	25.5	26.5	23.6	21.29	21.6	19.1	12.82	12.75	9.23
Average ratings of flours	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3	2	1	3	1	2	3

albumin was used. The test will be more popular with the members if fresh albumin is specified, as it gives a better cake, and one that can be used for demonstration purposes as well as test purposes.

COLLABORATOR C. Flour A appeared to be an over-treated flour or a very old flour from the soggy, crumbly texture of the cakes and from its pH, 5.0.

Flour B appeared to be a very fine cake flour not only from the results of all of the baking tests reported herein but also as the result of our regular baking procedure.

Flour C is too high a protein flour as well as too long an extraction to ever make a very good cake flour. However, it could be improved with an additional treatment of chlorine which, of course, would lower its pH.

The dried albumin had a very strong odor which tended to carry through into the cake. (A cake should be good enough to eat.) The cakes baked with the dried albumin were decidedly poor cakes in all cases. The frozen eggs produced nearly as good results as the fresh eggs. However, the frozen eggs did not seem to be as uniform as the fresh eggs. No advantage of the frozen eggs over the fresh eggs was found and the frozen eggs are hard to handle.

COLLABORATOR D. These tables give a most excellent picture of work done by members of your committee, and show definitely either fresh or frozen albumin may be used in making a test baking. Because these reports show little variation between fresh and frozen albumin and both are commonly used in the bake-shop, I believe both should be included. Due to the fact that dry albumin is little used and due to the findings of serious variations in dry albumin from different manufacturers and even from the same manufacturer that would materially affect the evaluation of the results, I believe these results would justify recommendations that dry albumin be eliminated from the tests.

COLLABORATOR E. The three flours were found to be in the same order regardless of the type of albumin. However, the use of fresh or frozen albumin in the formula is recommended.

Tests with Self-Rising Flours

The 1934–35 Self-Rising Flour Committee recommended that the collaborative baking work be continued along the lines followed by the committee during the previous year, in order to further test the reliability of the baking test. This committee also indicated that (*a*) scoring against a standard would be desirable; (*b*) determination of the variables in the individual score items should be ascertained, and (*c*) that standardization in the method of scoring should be worked on in order to develop more reliable methods of determining the scoring items outside of volume, *i.e.*, ultimately to express these scores numerically.

The reorganization of the committee during the year with the withdrawal of two collaborators, and the resulting late start, made an attempt to work on all phases of the problem impossible. It was, therefore, decided to confine the work to a determination of the degree of variation in the existing method of scoring.

Accordingly, there was distributed to each collaborator a short patent, soft wheat, self-rising flour to be used as a standard for scoring purposes. There were also distributed 4 samples of another grade of self-rising flour, with instructions that these 4 samples should be baked against the standard and scored individually. The 4 flour samples were identical, but this fact was unknown to the collaborators.

It is only in some manner such as this that the technician making the bake can obtain any degree of variation in such scores as flavor, color, and tenderness.

A sixth sample of a self-rising, clear flour was likewise included among the flours distributed.

The analyses of the plain, soft wheat flours used in these baking tests were as follows:

	Standard flour No. 1	Flour labeled Nos. 2, 3, 5, 6	Clear flour No. 4
Protein,[1] %	8.6	8.6	10.1
Moisture, %	12.0	12.3	12.5
Ash[1]	.313	.371	.629
pH	5.4	5.6	5.7

[1] Based on 15.0% moisture.

Prior to being sent out, the six flours were mixed with self-rising ingredients according to the 1935 formula, as follows:

Flour (15.0% moisture basis), grams	227.7
Sodium bicarbonate, grams	3.4
Monocalcium phosphate, grams	4.3
Salt, grams	4.6
	240.0

The collaborators were instructed to bake the flours according to the procedure given in the 1933–34 Committee report (Cereal Chem. **12**: 162) to include the 1935 revisions which consist of an increase in the size of the dough batch to 8 biscuits, making one extra biscuit available while hot for use in scoring the flavor, and in accordance with the following formula:

Flour (self-rising), 15.0% moisture basis, grams	240
Hydrogenated shortening (Crisco), grams	30
Distilled water	Variable
Temperature of dough	20° C.

The attached score card was used for scoring the biscuits. (Revised from Cereal Chem. **10**: 636, including 1935 changes.)

The results of the baking tests are recorded in Tables V, VI, and VII.

Recommendations

In the collaborative tests made by five collaborators, the majority of the findings confirmed the extensive bakings made in the comparison of fresh, frozen, and dried albumin, as presented in the paper by W. E. Stokes.

TABLE V

Summary of Total Scores Given by Several Collaborators after Testing Flours No. 1, 2, 3, 4, 5, 6

	Self-rising flours number					
Collaborator	1	2	3	4	5	6
A	100	95.25	91.25	85.15	93.0	93.25
B	100	90.14	91.29	85.0	90.43	96.91
C	100	95.0	95.90	89.2	94.7	94.7
Average score	100	93.46	92.81	86.45	92.71	94.95

TABLE VI

Table Showing Differences between Individual Scores and the Average Score, 93.48, of Flours 2, 3, 5, 6

Collaborator	Flour number				Mean difference	Individual average	Difference from total average
	2	3	5	6			
A	+1.77	−2.23	−0.48	−0.23	1.18	93.18	−0.3
B	−3.34	−2.19	−3.05	+3.43	3.00	92.18	−1.3
C	+1.52	+2.42	+1.22	+1.22	1.60	95.08	+1.6
Mean difference	2.21	2.28	1.58	1.63	1.92	Average 93.48	

Therefore, due to the variables found, it is recommended that dried albumin be eliminated and fresh or frozen albumin be used officially in the A. A. C. C. Basic Cake Baking Method.

The committee recommends for consideration the possibility of using the whole egg or the egg yolk in a formula designed to give a product similar to the pound cake type, and check the flours used in this test with the present A. A. C. C. baking test to determine whether a supplementary baking procedure is necessary for the proper evaluation of flours.

That type photographs illustrating grain, symmetry, and volume be selected from the work which has been presented and used next year in the collaborative work of the committee for the purpose of eliminating variables in the scoring of these items.

Certain variables have been reported in the use of hydrogenated shortening which affect volume. We recommend that this study be continued by this committee.

Last year the Committee on Self-Rising Flours baked 3 flours of definitely different grades which were submitted under code and on which the collaborators agreed in the relative position of the flours on quality.

TABLE VII

COMPARISON OF THE TOTAL AVERAGE SCORES ON INDIVIDUAL ITEMS WITH LIKE INDIVIDUAL AVERAGES OBTAINED BY EACH COLLABORATOR

	Total average scores				Collaborator A's scores				Collaborator B's scores				Collaborator C's scores			
	Average score	Low score	High score	Maximum variation	Average score	Low score	High score	Maximum variation	Average score	Low score	High score	Maximum variation	Average score	Low score	High score	Maximum variation
Grain	8.89	8.25	9.75	1.50	8.81	8.25	9.75	1.50	8.62	8.50	9.00	0.50	9.25	9.00	9.50	0.5
Tenderness	9.41	9.00	10.75	1.75	9.94	9.00	10.75	1.75	9.25	9.00	10.00	1.00	9.00	9.00	9.00	0.0
Flavor																
Flour	9.64	9.00	10.00	1.00	9.25	9.00	9.25	0.25	9.62	9.50	10.00	0.50	10.00	10.00	10.00	0.0
Eating	8.82	8.00	10.00	2.00	8.93	8.00	9.75	1.75	8.50	8.00	10.00	2.00	9.00	9.00	9.00	0.0
Crumb color	16.98	15.00	18.00	3.00	16.75	15.00	18.00	3.00	16.50	16.00	17.00	1.00	17.65	17.00	18.00	1.0
Volume	39.74	38.93	40.70	1.77	39.50	39.00	40.00	1.00	39.51	38.90	40.20	1.30	40.20	39.50	40.70	1.2
Total	93.48	90.14	96.91	6.77	93.19	91.25	95.25	4.00	92.19	90.14	96.91	6.77	95.08	94.70	95.90	1.2

1934–35 COMMITTEE ON METHODS OF TESTING SELF-RISING FLOURS

Score Card for Biscuits

Subject: _____ Flour: _____

_____ From: _____

Room temperature _____ _____

Oven time _____ _____

Oven temperature _____ _____

Formula:

Flour, *Gms.* _____

Shortening, *Gms.* _____

Soda, *Gms.* _____

Monocalcium phosphate, *Gms.* _____

Salt, *Gms.* _____

Distilled water, *Cc.* _____

Absorption, *Pct.* _____

pH of biscuit _____

Oven loss, *Pct.* _____

Specific volume (basis weight of dough) _____

Score:

		Perfect	*Faults*
Grain: Size and uniformity of cells	10		
Tenderness	10		
Flavor: Flour quality	10	Normal	Wheaty, starchy lacking
Flavor: Eating quality	10	Tender, friable	Doughy, dry, mealy, tough
Crumb color	20		
Volume *	40		
Total	100		

Penalty _____ Reasons: _____

Final score _____

Remarks: _____

* "Volume" is the term representing the "specific volume on basis weight of dough," to which is affixed a score value, 40, on the basis of a standard.

This year as a possibly more critical test of the ability of the committee to grade self-rising flours by the biscuit baking procedure, the collaborators were supplied with the 4 samples noted.

In this series of bakings it is not indicated from the score of the collaborators that they reached an agreement that the 4 samples represented the same flour. The maximum variations in the total score of the collaborators are greater than the committee believes they should be.

The average deviation in scoring these flours appears to be 1.92. Many of the items scored depend on personal judgment with no well defined standards available for comparison.

The difference from the average score indicates that A is average in his opinion of the flour, B tends to score lower, and C tends to score higher.

It may be observed from the maximum variations that there is least agreement in crumb color, eating quality, and tenderness, and this suggests that these points be worked on by the collaborators in an endeavor to come to closer agreement.

It appears that possibly four replicate bakes would be necessary for a truly representative score to be obtained. This again emphasizes the lack of a standard for each item, *i.e.*, a lack of a clear conception of how much to devaluate objectionable features.

STUDY ON STANDARDIZATION OF EGG WHITE IN A. A. C. C. WHITE CAKE FORMULA FOR TESTING SOFT WHEAT FLOURS[1]

W. E. Stokes and Laura K. Track

Royal Baking Powder Company, Brooklyn, New York

(Read at the Annual Meeting, June 1936)

In the paper read by Dr. Glabau at the Denver meeting of the A. A. C. C., he stated, "The full producing volume of the egg albumin seems to be the one property that has greatest bearing upon the results obtained when the albumin is used in the official cake baking test." Dr. Glabau further reported that of the one hundred eight samples of dried egg albumin obtained from manufacturers in this country and China, he found no two samples alike and that "—these variations are reflected in the finished cake."

The above findings were confirmed by bakings in our laboratory. When our bakings were started to compare dried albumin, frozen and fresh egg whites each month for one year, the same egg albumin was used in the series of tests until March. It was found, however, that too small an amount had been put aside, and it was necessary to obtain a fresh supply. The fresh sample came from the same source of supply (which Dr. Glabau had found to be more uniform than others), yet a very decided difference in our bakings with the fresh supply was found. It is to be noted that the volumes of our bakings with dried egg albumin in the last two series of tests are greater than those previously reported. This can be seen from Table I, column 2.

In the experiments, comparative bakings were made with dried albumin, frozen egg whites and so-called "day-old" eggs. The fresh eggs were taken from three chain stores and were purchased on the day these stores obtained delivery. The bakings were made in the third week of each month starting in June 1935 and completed in May 1936. Information was sought to determine if fresh eggs would necessarily show variation due to seasonal change and if there was any material difference in this regard between frozen and fresh eggs.

The frozen eggs were obtained from six different breaking plants located in different states. It was desired also to determine whether

[1] Subcommittee report, 1935–36 Committee on Cake Baking Tests and Self-Rising Flours.

TABLE I

SUMMARY OF MONTHLY AVERAGE CAKE VOLUMES COMPARING DIFFERENT TYPES OF EGG ALBUMIN

	Frozen whites	Dry albumin	Brand of eggs			Average for fresh whites
			S	M	R	
1935	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*	*Cc.*
June	826	789	840	820	815	825
July	826	788	820	840	839	833
August	829	788	863	845.5	845	851
September	827	784	845	841	838	841
October	834	798	849	846	831	842
November	837	787	861	826	844	844
December	827	783	829	829	812	820
1936						
January	828	787	824	822	838	828
February	829	778	829	841	827	832
March	822	786	832	828	821	827
April	829	808	824	829	825	826
May	818	811	829	833	836	833
Average for 12 months	828	790	841	833	836	833
Maximum volume, *Cc.*	837	811	863	846	845	851
Minimum volume, *Cc.*	818	778	820	820	812	820
Variation, *Cc.*	19	23	43	26	33	31

there might be differences in the frozen eggs obtained from different localities.

Our findings show that there was little change from month to month throughout the year in fresh eggs of best quality.

Frozen egg whites were found to be remarkably uniform and equal in value in this regard from all the breaking plants tested.

Day-old eggs were found to give a slightly larger average volume (but not more than 1% larger on the average through the year's test) than the frozen whites.

There was less uniformity among the three lots of fresh eggs obtained from different chain stores than with frozen eggs.

The average cake volume using frozen egg whites approximated the average cake volume using fresh whites over the 12-month period within 0.6%. The average cake volume when using dried egg albumin was 5.16% lower than the cake volume when using fresh egg whites.

The symmetry, crust characteristics, texture, grain, and color of the cakes made with frozen whites more nearly approximated those made with fresh whites than did those made with dry albumin. The results of the cake tests made with frozen whites are more easily translated into

TABLE II

COMPARATIVE CAKE VOLUMES OBTAINED WITH SEVERAL BRANDS OF GRADE "A" EGGS (WHITES ONLY), AND FROZEN EGG WHITES AND DRY ALBUMIN, RECONSTITUTED

June, 1935								July 1935								August, 1935								
Date	Temperature		Cake volume					Date	Temperature		Cake volume					Date	Temperature		Cake volume					
	Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites			
					S	M	R						S	M	R						S	M	R	
	°C.	°C	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.	
24th	24	23	829	—	842	—	—	25th	28	25	821	788	—	—	—	27th	25	23	816	788	—	—	—	
24th	24	22	827	782	—	—	—	25th	27	23	826	—	—	842	—	27th	25	23	816	—	—	845	—	
25th	27	24	825	—	—	807	—	25th	26.5	24.5	818	—	—	—	834	28th	25	23	836	—	—	846	—	
26th	29	24	824	—	—	—	815	26th	28	23	837	—	—	—	844	28th	26	23	839	—	—	—	831	
26th	29	24	821	—	—	832	—	26th	26	23	835	—	—	837	—	28th	26	23.5	825	—	877	—	—	
27th	30	23.5	834	796	—	—	—	26th	25	23	822	—	817	—	—	29th	28	24	831	—	857	—	—	
27th	27.5	23.5	819	—	—	—	814	27th	25.5	23	826	—	822	—	—	29th	26	22.5	834	—	—	—	863	
27th	28.5	24.5	817	—	839	—	—									30th	26	23	824	—	—	—	842	
																30th	26	22.5	839	—	856	—	—	
Average cake volume, *Cc.*			826	789	840	820	815	Average cake volume, *Cc.*			826	788	820	840	839	Average cake volume, *Cc.*			829	788	863	845.5	845	

TABLE III

COMPARATIVE CAKE VOLUMES OBTAINED WITH SEVERAL BRANDS OF GRADE "A" EGGS (WHITES ONLY), AND FROZEN EGG WHITES AND DRY ALBUMIN, RECONSTITUTED

September, 1935								October, 1935								November 1935								
Date	Temperature		Cake volume					Date	Temperature		Cake volume					Date	Temperature		Cake volume					
	Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites			
					S	M	R						S	M	R						S	M	R	
	°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.	
25th	26	22.5	831	—	—	—	841	22nd	23	22	833	813	—	—	—	26th	18	22	830	—	—	—	840	
25th	26	23	828	—	851	—	—	22nd	23	22	816	—	—	813	—	27th	18	22	844	—	—	816	—	
26th	22	23	830	—	—	818	—	22nd	23	22	839	—	—	862	—	27th	18	23	839	—	856	—	—	
26th	23.5	24	822	—	—	—	835	23rd	23	22	838	—	—	—	838	29th	18	23	836	—	—	—	848	
26th	23	23	828	—	832	—	—	23rd	23	22	833	—	848	—	—	29th	16	23	—	—	866	—	—	
26th	26	22	822	—	—	847	—	23rd	23	23	827	—	—	—	822	30th	18	23	839	787	—	—	—	
30th	17	19	831	—	853	—	—	24th	20	22	842	—	—	864	—	30th	18	23	834	—	—	836	—	
30th	22.5	21	833	784	—	—	—	24th	20	21	836	—	856	—	—									
30th	21	20.5	820	—	—	858	—	24th	24	22	842	—	—	—	844									
								25th	18	19	838	—	843	—	—									
								25th	23	22	828	783	—	—	—									
Average cake volume, *Cc.*			827	784	845	841	838	Average cake volume, *Cc.*			834	798	849	846	831	Average cake volume, *Cc.*			837	787	861	826	844	

TABLE IV

COMPARATIVE CAKE VOLUMES OBTAINED WITH SEVERAL BRANDS OF GRADE "A" EGGS (WHITES ONLY), AND FROZEN EGG WHITES AND DRY ALBUMIN, RECONSTITUTED

December, 1935								January, 1936								February, 1936							
Date	Temperature		Cake volume					Date	Temperature		Cake volume					Date	Temperature		Cake volume				
	Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites		
					S	M	R						S	M	R						S	M	R
	°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.
26th	18	23	821	—	831	—	—	23rd	14.5	21	828	787	—	—	—	25th	18	22	824	778	—	—	—
26th	18.5	22	825	—	—	822	—	23rd	14	19	828	—	—	—	841	25th	19.5	22	834	—	812	—	—
26th	20	23	838	783	—	—	—	24th	15	24	823	—	—	—	843	25th	19.5	21	839	—	—	—	830
27th	16	20	830	—	—	—	818	24th	15	24	839	—	—	841	—	25th	18.5	21.5	822	—	—	—	831
27th	19	22.5	828	—	—	837	—	27th	17	21	840	—	—	—	841	26th	20	21.5	831	—	—	828	—
27th	19	22.5	826	—	828	—	—	27th	17	23	824	—	—	813	—	26th	19	21.5	827	—	839	—	—
27th	10	21	825	—	—	—	804	27th	17	23	824	—	826	—	—	26th	20	21.5	822	—	—	—	821
30th	18	22	821	—	—	—	815	27th	18	22	823	—	—	—	819	26th	20	21.5	836	—	—	851	—
30th	17	22	825	—	—	828	—	24th	16	23	821	—	822	—	—	27th	21	22	834	—	836	—	—
30th	17	22	833	—	828	—	—									27th	21	21.5	830	—	—	844	—
Average cake volume, *Cc.*			827	783	829	829	812	Average cake volume, *Cc.*			828	787	824	822	838	Average cake volume, *Cc.*			829	778	829	841	827

TABLE V

COMPARATIVE CAKE VOLUMES OBTAINED WITH SEVERAL BRANDS OF GRADE "A" EGGS (WHITES ONLY), AND FROZEN EGG WHITES AND DRY ALBUMIN, RECONSTITUTED

March, 1936								April, 1936								May, 1936							
Date	Temperature		Cake volume					Date	Temperature		Cake volume					Date	Temperature		Cake volume				
	Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites				Room	Batter	Frozen whites	Dry albumin	Fresh egg whites		
					S	M	R						S	M	R						S	M	R
	°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C.	Cc.	Cc.	Cc.	Cc.	Cc.		°C.	°C,	Cc.	Cc.	Cc.	Cc.	Cc.
24th	18.5	21.5	809	—	—	833	—	27th	18	21	825	810	—	—	—	19th	25	23	818	—	824	—	—
24th	18.5	22	826	—	—	833	—	27th	19	21	828	805	—	—	—	19th	25	22	818	—	—	831	—
25th	20	20	808	785	—	—	—	28th	19	21.5	828	810	—	—	—	19th	24	22	819	—	816	—	—
25th	19	21	828	—	847	—	—	28th	19	22	830	—	—	833	—	19th	25	22	817	—	—	825	—
26th	19	22	826	785	—	—	—	28th	18	21.5	831	—	—	825	—	20th	19	21.5	818	—	—	—	833
26th	20	21	838	—	—	818	—	29th	23	22	829	—	846	—	—	20th	19	22.5	822	—	—	842	—
26th	21	21	824	—	817	—	—	30th	26	24	832	—	—	—	825	20th	20	22.5	818	811	—	—	—
27th	21	21	820	788	—	—	—	30th	25	24	818	—	—	—	812	20th	20	22.5	815	—	—	—	835
27th	21	22	823	—	—	—	830	30th	26	22	832	—	—	—	843	21st	20	22	815	—	—	—	840
27th	21	22	815	—	—	—	812	30th	26	23.5	834	—	—	—	819	21st	21	22.5	822	—	846	—	—
								30th	19	21.5	823	—	825	—	—								
Average cake volume, *Cc.*			822	786	832	828	821	Average cake volume, *Cc.*			829	808	824	829	825	Average cake volume, *Cc.*			818	811	829	833	836

practical production terms with respect to sugar, liquid, and shortening tolerances as well as tenderness and silkiness of crumb, than is possible when using dried egg albumin.

In addition, the more uniform cake volume results obtained with frozen whites over a long period offer advantages over the fluctuating cake volumes obtained when using fresh egg whites.

The comparative baking data have been summarized in Table I. The data obtained throughout the year are to be found in Tables II to V inclusive.

Data were also accumulated relative to the monthly price of grade "A" eggs, and the yield of whites per dozen during the period of June 1935 to May 21, 1936. These data will be found in Table VI.

TABLE VI

PRICE OF GRADE "A" EGGS AND YIELD OF WHITES PER DOZEN

Brand	S		M		R	
	Price per dozen	Amount of whites	Price per dozen	Amount of whites	Price per dozen	Amount of whites
1935	*Cents*	*Cc.*	*Cents*	*Cc.*	*Cents*	*Cc.*
June	39	408	39	390	39	386
July	43	398	43	401	43	378
August	47	415	47	380	47	370
September	49	396	51	383	51	389
October	55	395	53	363	57	395
November	51	371	53	381	49	369
December	43	404	49	381	49	412
1936						
January	39	373	41	355	41	394
February	45	404	43	386	47	397
March	39	362	39	363	39	385
April	39	361	39	388	39	371
May	39	364	39	376	39	385
Maximum	55	415	53	401	57	412
Minimum	39	361	39	355	39	369
Variation	16	54	14	46	18	43

FACTORS AFFECTING THE BASIC CAKE FORMULA

The Effect of Different Kinds of Shortening on the Characteristics of the Cake as Determined by the A. A. C. C. Procedure [1]

H. R. FISHER

Twin Falls Flour Mills, Twin Falls, Idaho

Committee work of the past has shown the advisability of standardizing the various ingredients in the basic cake baking formula. The object of this paper is to discover whether or not the shortening used is a source of variation. In order to investigate this point three high grade, well known, hydrogenated shortenings known to have slightly different properties, were chosen for a series of bakings using the basic method. It was believed that the specific gravity of the batter would serve to illustrate any differences which might exist in the abilities of the several shortenings to cream up and emulsify in the formula. To further illustrate the leavening action of the shortenings, the chemical leavening was lowered in the basic formula as shown in Table I. The specific volume of the cakes was taken as an index to the leavening.

TABLE I

Shortening	Cream of tartar	Soda	Specific gravity of batter	Specific volume of cake
A	6	3	1.17	2.54
A	5	2½	1.16	2.17
B	6	3	.94	2.96
B	5	2½	.90	2.85
B	4	2	.92	2.56
C	6	3	1.02	2.81
C	5	2½	1.02	2.78
C	4	2	1.01	2.58

A highly bleached cake flour was chosen to minimize the tendency of the cakes to fall due to a possible excess of leavening.

The results of the investigation have been tabulated and are shown as Table I.

There was quite a marked difference in the appearance of the bat-

[1] Subcommittee report, 1935–36 Committee on Cake Baking Tests and Self-Rising Flours.

ters. Shortening "A" gave a thin, watery batter, while shortening "B" gave a batter so stiff that it was difficult to pour. Shortening "C" was between "A" and "B" in appearance and consistency.

Cakes made from shortening "A" were very poor, being tough and harsh; their grain was uneven and open. Lowering the soda a half-gram and the cream of tartar 1 gram lowered the specific volume of the cake from 2.54 to 2.17. The cake seemingly did not rise in the oven at all. Cakes made with the basic formula and with this shortening seem to depend almost entirely on the chemical leavening to raise the cake.

This came somewhat as a surprise as all three shortenings had made creditable cakes when the sugar and shortening were creamed together as in the ordinary commercial methods.

In the case of shortenings "B" and "C" the cakes had a higher specific volume using one-third less chemical leavening, than those cakes made with shortening "A" and the full amount of chemical leavening.

Shortening "B," using 2.5 g. of soda and 5 g. cream of tartar, compared favorably with shortening "C" using the full amount called for in the formula.

It was quite obvious shortening "A" is not suitable for the basic method. Shortenings "B" and "C" are both suitable but show considerable variation.

Conclusions

The choice of shortening for use in the basic method is a source of considerable variation. Shortening should be studied with a view to standardization for the A. A. C. C. Cake Baking Method.

REPORT OF THE 1935–36 COMMITTEE ON TESTING BISCUIT AND CRACKER FLOURS

WALTER REIMAN, *Chairman*

Kroger Food Foundation, Cincinnati, Ohio

(Read at the Annual Meeting, June 1936)

With the possible exception of the viscosity test, the methods used for evaluating biscuit and cracker flours are much the same as those employed in testing other wheat products. These methods are the chemical analyses for moisture, protein, and ash contents, a baking test, and the determination of the hydrogen-ion concentration of the product. The problem of the Committee on Testing Biscuit and Cracker Flours lies not only in the intelligent use of the methods mentioned above and the development of new tests, but also in correlating and properly interpreting the results obtained from these tests when applied to biscuit and cracker flours.

The work of the 1934 and 1935 Committees on Testing Biscuit and Cracker Flours (see Bohn, 1934, 1935) showed such consistently good results for the viscosity test as an index of strength for standard patent flours that tentative viscosity standards were suggested. On the other hand, it was concluded that the baking test [1] was of less collaborative value because the comparative test loaves (by description and volume at least) were quite dissimilar. The report of the 1935 committee (see Bohn, 1935) recommended that in future work, this committee should give attention to flours of the same strength as determined by the viscosity test but widely different in protein content and *vice versa*.

The work of the present committee was directed toward carrying out the recommendation of its predecessor and toward more accurately determining the value of the baking test for the purpose of evaluating biscuit and cracker flours. A start has also been made to determine the value of a knowledge of the hydrogen-ion concentration of flours to be used in biscuit and cracker production.

Plan of Procedure

The members of the committee entered into a series of collaborative tests to fulfill the aforementioned purposes. Three commercially milled soft winter wheat flours were selected as specimens for the collaborative

[1] For description see Cereal Chemistry **11**: 662–663.

work. Two of these flours had approximately the same ash and protein contents but different viscosities; the third flour was lower in ash and protein contents and also viscosity than either of the other two flours. The three flours were sent to the collaborators for testing three separate times, but were coded so as not to reveal that identical flours were being tested in each series.

On the first series of tests the committee members were asked to bake duplicate loaves from each of the flours, using method A outlined below, and to determine the pH of the flour. One set of loaves was to be scored by the collaborator and the other sent to the chairman of the committee to be photographed.

For the second series of tests the collaborators were asked to determine the moisture, ash, and protein contents of the flours and to measure their viscosity, and on this basis to again classify the three flours.

In the third series of tests the members of the committee were asked to bake the flours, using method B outlined below, and to score and handle the loaves as in the first series of tests. From their results on each series of tests the collaborators were asked to classify the flours according to the commercial use for which they thought them to be best suited. A questionnaire was also submitted to the collaborators for the purpose of obtaining opinions as to the significance and interpretation given to the various tests involved in this work.

Finally, three sets of loaves were baked from the flours at the Kroger Food Foundation, according to method B, and sent to three members of the committee who were asked to score the loaves and to classify the flours by using the same methods of interpretation which they applied when evaluating the loaves baked by themselves.

Materials and Methods of Study

Materials

The origin, type of milling, and treatment of the three flours used in this investigation, and which shall be designated as No. 1, No. 2, and No. 3, were as follows:

Flour No. 1.—This flour was milled of Ohio soft red winter wheat averaging approximately 10% protein. It was milled to a 95% extraction and was unbleached.

Flour No. 2.—This flour was milled from Ohio mixed wheat which averaged 60% white and 40% red wheat varieties. Since the mineral content of white wheat runs several hundredths of a percent higher than red wheat grown under the same conditions, this flour was milled to an 85% extraction. It was unbleached.

Flour No. 3.—This flour was milled exclusively of Michigan white

wheat which contained less than 10% of red varieties. This flour was milled to 65% extraction and was chlorine bleached to a pH of approximately 5.2.

None of these flours received any additions of special substances to lower the viscosity nor were they heat-denatured or treated in any way, other than No. 3 which received a normal dosage of chlorine.

Baking Methods

Method A.—Baking method A is the Werner test as described in Cereal Chemistry, **5:** p. 158 (March 1928) with the following variations: a 2-hour fermentation time with 1 punch only at 1 hour and 35 minutes; 3 g. of sugar instead of 2.5 g.; baking temperature 420° to 430° F. for 25 minutes; a rotary oven used, if possible.

Method B.—Baking method B is the Werner test as given in Cereal Chemistry, **5:** p. 158 (March 1928) with the following variations: in mixing, regulate temperature of ingredients so that dough comes from mixer at 80° F.; in the molding and panning procedure, after pounding (as illustrated in Figure 2, Cereal Chem. **5:** 160) cut dough from board and fold in half, putting side of dough which was next to the board on the inside. Again fold dough in half and pound with heel of hand and mold (Figure 3, *ibid.*); ferment at 84° F.; proof 60 minutes under same conditions as for fermentation; bake 25 minutes at 420° to 430° F.

The following terminology should be used in describing the test loaves:

Oven spring (expansion in oven)—large, medium, small
Break and shred—smooth, shell top, torn, very ragged, none
Crust color—dark, medium, pale
Grain—very coarse, coarse, close and round, close and elongated
Color of crumb—white, creamy bright, creamy, gray, dull
Volume—cubic centimeters
Absorption—percent

Viscosity Determination

Weigh out 20 g. of flour—15% moisture basis. Transfer to a mortar and add 40 cc. distilled water. Stir with a small spatula until all the flour particles are wetted and then rub into a smooth paste with a pestle. Add 30 cc. more of distilled water, stir, and then pour the suspension into the viscosimeter bowl. Rinse the mortar with two 15 cc. portions of distilled water and add the rinsings to the suspension in the bowl. A total of 100 cc. of distilled water should be used. Start the viscosimeter, stir the suspension with the bob and then make an initial reading. Add 1 cc. of 1.0 N lactic acid, stir the suspension thoroughly with the bob and make another reading. In a like manner add 6.0 cc. more lactic acid in 2 cc. portions making a reading after each acidulation.

Use a MacMichael viscosimeter, fitted with a No. 30 wire and 6.0 cm. bob, and operate at 12 r.p.m.

Hydrogen-Ion Concentration—pH

Weigh 10 g. of flour into a clean, dry Erlenmeyer flask and add 100 cc. of cool but recently boiled distilled water. Make a suspension of the flour in water. Place in a thermostat at 25° C. for 30 minutes, keeping the flour in suspension by occasional shaking. At the end of this time allow the flask to stand quietly for 10 minutes and then decant the supernatant liquid through a hardened, dry,

folded filter. Re-filter the first 5 cc. to come through and determine the pH of the remaining filtrate either colorimetrically or electrometrically.

Experimental Results

Tables I, II, and III record the analytical findings, the viscosity readings, and the pH results obtained on the three flours used in this investigation. The classifications assigned by the collaborators on the basis of the viscosity test are also given. Tables IV, V, and VI record the scores

TABLE I

CHEMICAL AND VISCOSITY READINGS OBTAINED BY THE COLLABORATORS WITH FLOUR NO. 1

Collaborator	Moisture	Ash as received	Ash at 15% H_2O	Protein as received	Protein at 15% H_2O	pH	Viscosity °MacM.		
							No acid	1 cc.	7 cc.
	%	%	%	%	%				
Pearl Brown	12.14	0.397	0.384	8.64	8.36	5.70	—	—	—
S. M. Jackson	11.70	.384	.370	8.67	8.34	6.44	8	25	48
J. Micka	11.10	.390	.373	8.80	8.41	—	—	—	50 [1]
C. O Oppen	11.20	.395	.378	8.83	8.45	5.80	8.5	27	50
E. L. Ulrey	11.50	.400	.384	8.76	8.41	6.00	12	33	53
Kroger Foundation	9.51[2]	.402	.377	8.94	8.40	6.03	8	30.5	52.5
Mean	11.53	.395	.378	8.74	8.40	5.99	9.0	29.0	51
Maximum	12.14	.402	.384	8.94	8.45	6.44	12.0	33	53
Minimum	9.51	.384	.370	8.64	8.34	5.70	8.0	25	48

[1] After addition of 5 cc. acid.
[2] Not included in average.

COLLABORATORS' CLASSIFICATION OF FLOUR NO. 1 FROM VISCOSITY DATA:

Pearl Brown: (Opinion based on average of viscosity readings reported by other collaborators.) "Might be more or less of a border line flour; *i.e.*, can be used for either sponging or doughing."

Jan Micka: "I consider this a weak sponge flour. Blended with a stronger flour in the sponge or a weaker flour in the dough it should give good results in cracker doughs."

C. O. Oppen: "An excellent cracker doughing flour."

Kroger Foundation: "A flour suitable for doughing purposes or for general use applied to hard-sweet goods. The strongest of the three samples.

of the loaves baked by the collaborators using methods A and B. These tables also include the classification of the flours by the collaborators based on the results obtained by means of each baking test. Figures 1, 2, 3, and 4 are photographs of the loaves baked from the flours by the collaborators using methods A and B respectively. Table VII summarizes the observations and classifications of the collaborators on loaves baked from the three flours in the cereal laboratory of the Kroger Food Foundation.

TABLE II

Chemical and Viscosity Readings Obtained by the Collaborators with Flour No. 2

Collaborator	Moisture	Ash as received	Ash at 15% H_2O	Protein as received	Protein at 15% H_2O	pH	Viscosity °MacM.		
							No acid	1 cc.	7 cc.
	%	%	%	%	%				
Pearl Brown	12.00	0.359	0.347	8.00	7.73	5.5	—	—	—
S. M. Jackson	11.60	.361	.347	8.05	7.74	6.38	7	17	25
Jan Micka	11.40	.380	.365	8.20	7.87	—	—	—	30 [1]
C. O. Oppen	11.10	.380	.363	8.21	7.85	5.8	8	21	32
E. L. Ulrey	11.10	.380	.363	8.36	8.00	6.0	10	23	30
Kroger Foundation	8.94[2]	.370	.345	8.32	7.76	5.84	7.5	19.5	27.5
Mean	11.45	.372	.356	8.17	7.83	5.90	8	20	29
Maximum	12.00	.380	.365	8.36	7.87	6.38	10	23	32
Minimum	8.94	.359	.345	8.00	7.73	5.50	7	17	25

[1] After addition of 5 cc. acid.
[2] Not included in average.

Collaborators' classification of flour No. 2 from viscosity data:

Pearl Brown: (Opinion based on average of viscosity readings reported by other collaborators.) "A flour for use in sweet goods."
Jan Micka: "A cracker dough flour or all around cookie flour."
C. O. Oppen: "A flour for high ratio cake, cookies, *etc.*"
Kroger Foundation: "A flour to be used with cookies or short breads. Weaker than flour No. 1 but stronger than flour No. 3."

TABLE III

Chemical and Viscosity Readings Obtained by the Collaborators Using Flour No. 3

Collaborator	Moisture	Ash as received	Ash at 15% H_2O	Protein as received	Protein at 15% H_2O	pH	Viscosity °MacM.		
							No acid	1 cc.	7 cc.
	%	%	%	%	%				
Pearl Brown	12.08	0.357	0.345	7.12	6.89	5.30	—	—	—
S. M. Jackson	11.70	.342	.329	7.21	6.94	6.33	3	9	13
J. Micka	11.20	.360	.345	7.20	6.89	—	—	—	15 [1]
C. O. Oppen	11.45	.357	.345	7.52	7.22	5.20	4.5	14	18
E. L. Ulrey	11.50	.360	.346	7.40	7.11	5.60	5	12	15
Kroger Foundation	9.03[2]	.362	.338	7.51	7.02	5.24	3.5	10	12
Mean	11.59	.356	.341	7.33	7.01	5.53	4	11	15
Maximum	12.08	.362	.346	7.52	7.22	6.33	5	14	18
Minimum	9.03	.342	.329	7.12	6.89	5.20	5	9	12

[1] After addition of 5 cc. acid.
[2] Not included in average.

Collaborators' classification of flour No. 3 from viscosity data:

Pearl Brown: (Opinion based on average of viscosity readings reported by other collaborators.) "Probable use questionable because of such low viscosity."
Jan Micka: "Too soft even for wire cut pieces. May be used if blended with a stronger flour."
C. O. Oppen: "Very soft, excellent cookie flour."
Kroger Foundation: "Another cookie and short bread flour. In comparison with flour No. 2, it should give greater spread with less sugar and shortening. It is the weakest of the three flours."

TABLE IV

Bread Scores Obtained by the Collaborators when Using Flour No. 1

	Loaves baked by method A				Loaves baked by method B			
Collaborator	Pearl Brown [1]	C. O. Oppen [2]	E. L. Ulrey	Kroger Foundation [3]	Pearl Brown [4]	C. O. Oppen [5]	E. L. Ulrey [6]	Kroger Foundation [7]
Oven spring	Large	Large	Large	Large	Large	Large	Medium	Large
Break and shred	Fairly smooth	Torn	Torn	Smooth	Fairly smooth	Very ragged	Torn	Torn
Crust color	Dark (good color)	Dark	Medium	Dark	Dark (very good)	Dark	Pale	Medium
Grain	Close, round	Close and round	Close and round	Coarse	Close, slightly elongated	Close and round	Close and round	Close and round
Color of crumb	Creamy (yellow)	Creamy	Creamy bright	Creamy	Creamy	Creamy bright	Creamy	Creamy
Volume, *cc.*		465	550	435		450	460	435
Absorption, %	48	47	56	56	48	48	51.5	55

[1] Pearl Brown: "A strong flour—sponge type."
[2] C. O. Oppen: "A cracker sponging flour."
[3] Kroger Foundation: "An unbleached flour suitable for use in cracker sponging work."
[4] Pearl Brown: "A strong sponge flour."
[5] C. O. Oppen: "Desirable for cracker doughing."
[6] E. L. Ulrey: "A cracker flour."
[7] Kroger Foundation: "An unbleached flour, the strongest of these three; should find its best application as a sponging flour."

TABLE V

Bread Scores Obtained by the Collaborators when Using Flour No. 2

	Loaves baked by method A				Loaves baked by method B			
Collaborator	Pearl Brown [1]	C. O. Oppen [2]	E. L. Ulrey	Kroger Foundation [3]	Pearl Brown [4]	C. O. Oppen [5]	E. L. Ulrey [6]	Kroger Foundation [7]
Oven spring	Medium +	Medium	Large	Large	Medium, large	Medium	Medium	Large
Break and shred	Slightly ragged	Ragged	Torn	Torn	Slightly shell ragged	Torn	Torn	Torn
Crust color	Medium	Medium	Medium	Medium	Medium	Medium	Pale	Medium to pale
Grain	Coarse, elongated	Close, elongated	Close, elongated	Close, round	Close, slightly elongated	Close, elongated	Coarse	Slightly coarse, close and round
Color of crumb	White	White	White	White	White	White	White	White
Volume, *cc.*		390	520	425		400	460	410
Absorption, %	48	44	54	55—	48	45	52	53

[1] Pearl Brown: "Also strong. Could be used in sponge or doughs. Probably would be better in doughs."
[2] C. O. Oppen: "A cracker doughing or cookie flour."
[3] Kroger Foundation: "A borderline case, wherein it might be used in cracker sponging and doughing and in certain types of sweet goods."
[4] Pearl Brown: "A fairly strong flour-dough type but could be used in sponges too."
[5] C. O. Oppen: "A flour desirable for cookies, cones, etc."
[6] E. L. Ulrey: "A cookie flour."
[7] Kroger Foundation: "Suitable for either cracker sponging or doughing, a borderline case; would give preference to its application to doughing and sweet goods."

TABLE VI

Bread Scores Obtained by the Collaborators when Using Flour No. 3

	Loaves baked by method A				Loaves baked by method B			
Collaborator	Pearl Brown [1]	C. O. Oppen [2]	E. L. Ulrey	Kroger Foundation [3]	Pearl Brown [4]	C. O. Oppen [5]	E. L. Ulrey [6]	Kroger Foundation [7]
Oven spring	Almost none	Small	Small (none)	Small	Practically none	Small	Small	Small
Break and shred	Very ragged	None	Very ragged	None	Scarcely any, rough	None	None	Shell topped
Crust color	Pale	Pale	Pale	Pale	Pale	Pale	Pale	Pale
Grain	Very coarse	Coarse	Very coarse	Very coarse	Coarse, uneven, round	Coarse	Very coarse	Very coarse
Color of crumb	Gray, dull	Creamy white, dull	Dull	Creamy, dull	Grayish	Dull	Creamy, white	Creamy, dull
Volume, *cc.*		320	330	385		330	380	315
Absorption, %	48	44	53	54—	48	42	52	52

[1] Pearl Brown: "Very weak. Doughs were mellow, not spongy or stretchy. Could possibly be used in certain types of soft cakes."
[2] C. O. Oppen: "For use in sweet goods requiring a very weak flour."
[3] Kroger Foundation: "Definitely a cookie flour with Michigan white wheat flour characteristics."
[4] Pearl Brown: "A mellow flour. Almost no tenacity. Certainly not a sponge or dough type. Of questionable value as a general cookie flour."
[5] C. O. Oppen: "A very weak flour. Desirable for cookies, vanilla wafers, etc."
[6] E. L. Ulrey: "A cake flour."
[7] Kroger Foundation: "Definitely the softest of the three flours. A cookie flour. Similar to those milled from Michigan white wheat or extremely soft Pacific Coast wheats."

TABLE VII

Collaborators' Scores on Loaves Baked at the Kroger Foundation

Flour	No. 1			No. 2			No. 3		
Collaborator	Pearl Brown [1]	C. O. Oppen [2]	E. L. Ulrey [3]	Pearl Brown [4]	C. O. Oppen [5]	E. L. Ulrey [6]	Pearl Brown [7]	C. O. Oppen [8]	E. L. Ulrey [9]
Oven spring	Fairly large	Medium	Medium	Medium	Medium	Medium	Small	Small	Small
Break and shred	Slightly torn	Smooth	Shell top	Torn	Torn	Very ragged	Shell top, rough	Torn	Very ragged
Crust color	Medium	Medium	Pale	Pale	Medium	Pale	Pale	Pale	Pale
Grain	Fairly close, elongated	Close and round	Close and round	Coarse, elongated	Close and elongated	Close and round	Coarse, round, elongated	Coarse	Coarse
Color of crumb	Creamy	Creamy	Creamy	Grayish white	White	White	White	Dull	Creamy bright
Volume, *cc.*		430	400		420	385		345	335

[1] Pearl Brown: "A sponge flour. Could be used in some doughs but is rather strong."
[2] C. O. Oppen: "A typical cracker sponging type flour."
[3] E. L. Ulrey: "A cracker sponging flour."
[4] Pearl Brown: "Dough flour."
[5] C. O. Oppen: "A cracker doughing and general cake flour."
[6] E. L. Ulrey: "A cracker doughing flour, which could also be used in certain types of sweet goods."
[7] Pearl Brown: "I still don't know what I'd really like to use that flour for if I were to have it to use in a shop."
[8] C. O. Oppen: "A typical cookie type flour."
[9] E. L. Ulrey: "A very soft flour to be used in cookies and cakes."

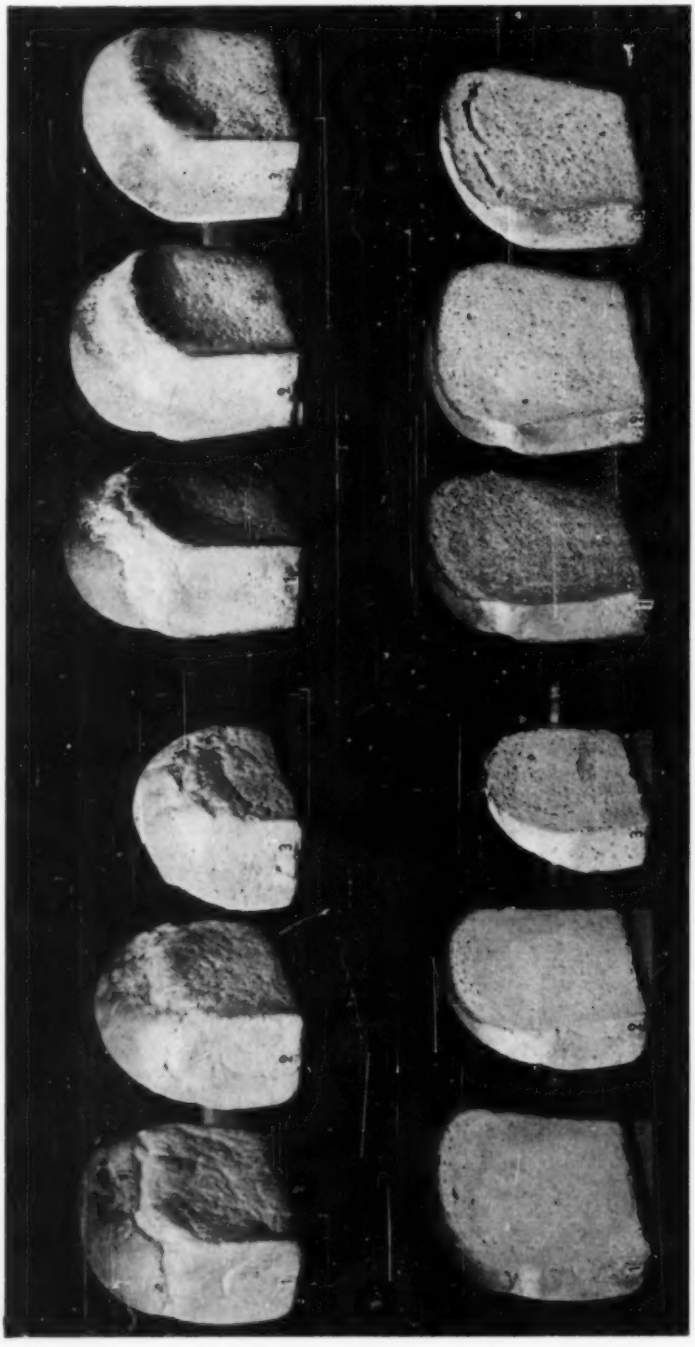

Figure 1. Loaves baked respectively by C. O. Oppen and at The Kroger Food Foundation using method A.

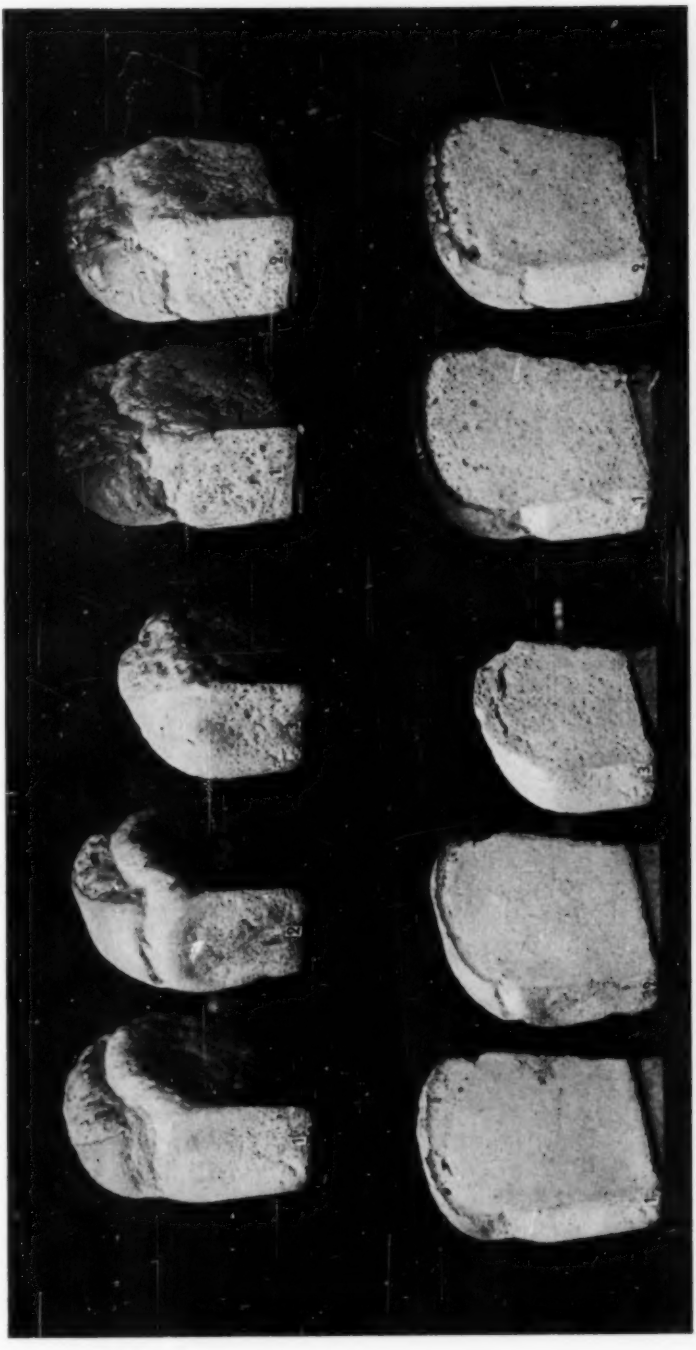

Figure 2. Loaves baked respectively by E. L. Ulrey and Miss Brown using method A.

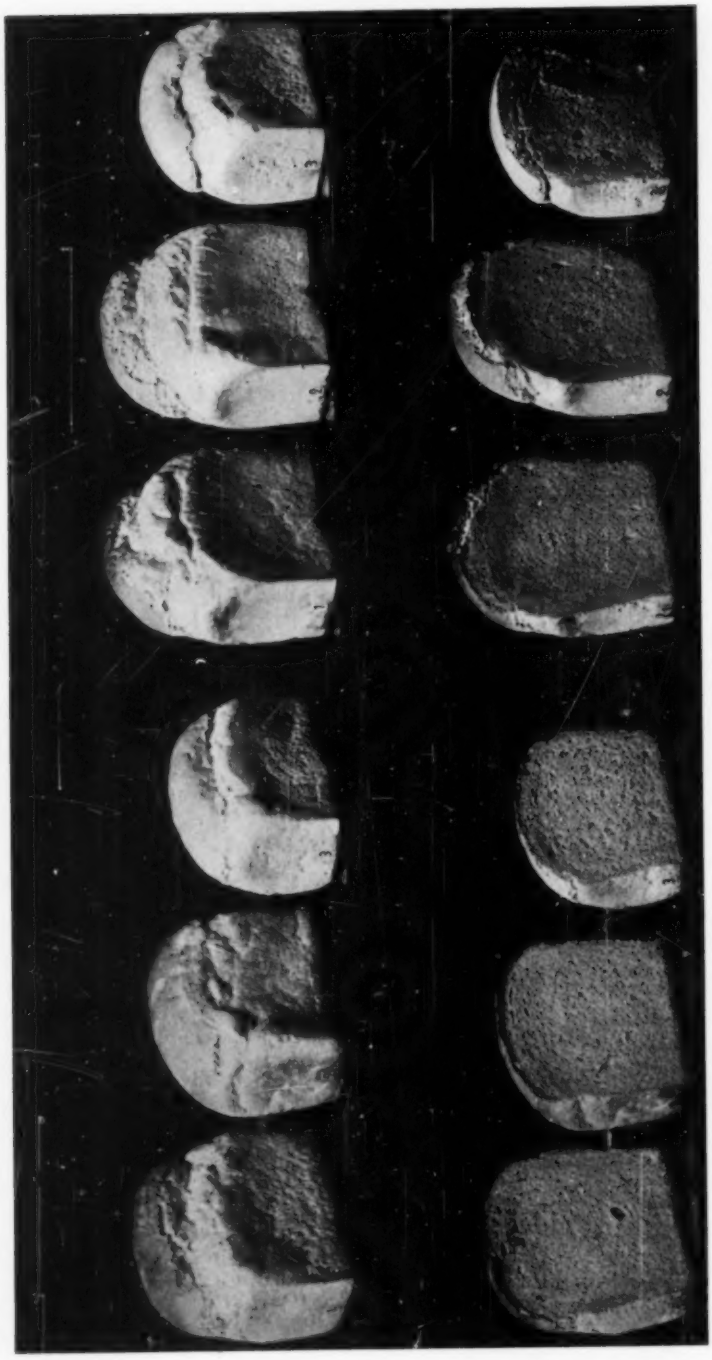

Figure 3. Loaves baked respectively by C. O. Oppen and at The Kroger Food Foundation using method B.

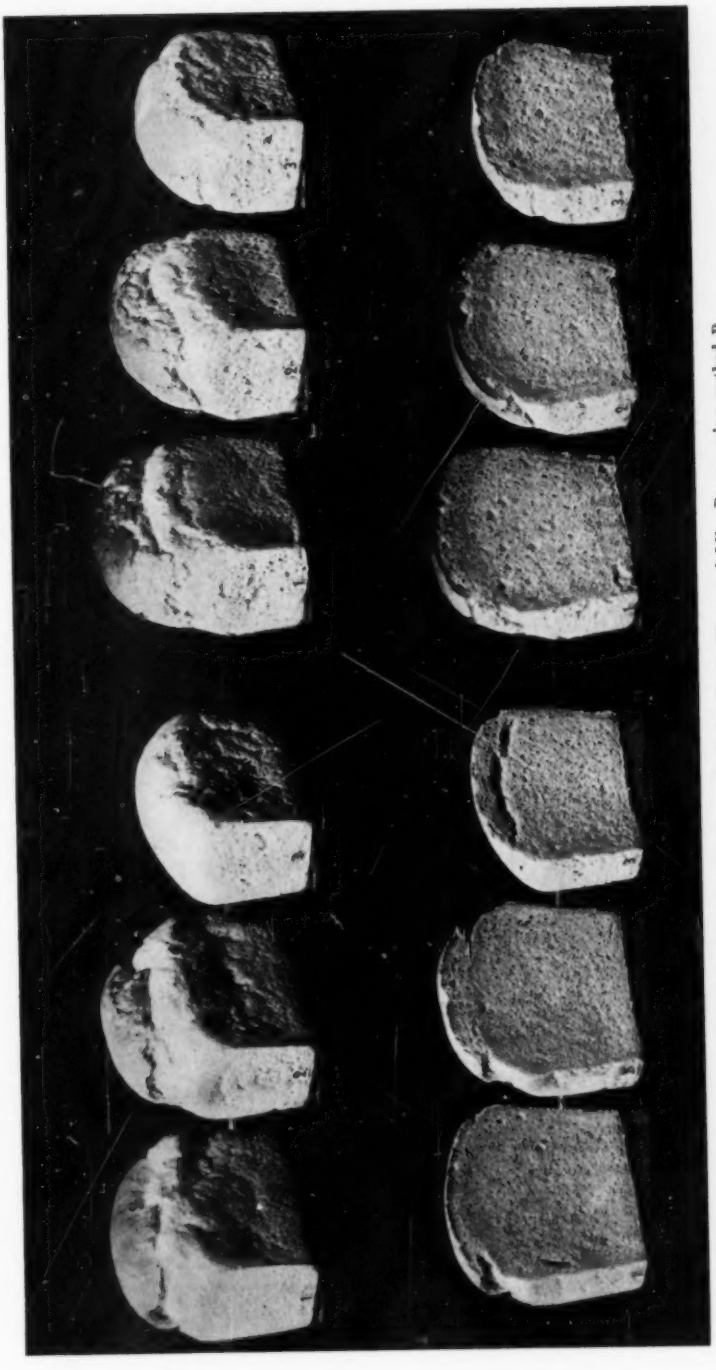

Figure 4. Loaves baked respectively by E. L. Ulrey and Miss Brown using method B.

Discussion of Results

Each committee member was asked to answer a set of questions relative to a few of the various tests being used by the committee as a whole for the purpose of evaluating biscuit and cracker flours. Below are given the questions followed by the answers of the committee members who collaborated in this work.

(1) Which of the tests, *i.e.*, baking, viscosity, or analytical, did you consider best for evaluating the three flours?

Pearl Brown: "The viscosity test probably gives the most information in the shortest time. I think the analytical data need to be considered with it however."

S. M. Jackson: "Probably no one of them by itself is sufficient."

J. Micka: "The analytical test combined with viscosity, for anybody who knows what kind of flours are used in actual baking operations, is sufficient. The origin of the wheat and location of the mill is very helpful information."

C. O. Oppen: "The viscosity test is the most valuable with the baking test a close second."

E. L. Ulrey: "The analytical data including the viscosity test."

Kroger Foundation: "The baking tests."

(2) In your opinion which of the two baking tests is the better for judging flours of this type?

(*a*) From the standpoint of convenience.

(*b*) Ability to differentiate between flours.

(*c*) Gives the most information about a flour.

Pearl Brown: (*a*) Method A.
(*b*) Method B.
(*c*) Method B perhaps. Especially with the stronger types of flour.

J. Micka: Any bread baking test may give some information on cracker flours as far as fermentation and enzymatic activity are concerned. However, I don't think that the bread baking test is a good test for cookies and biscuits.

C. O. Oppen: (*a*) Method A.

Kroger Foundation: (*a*) Method B.
(*b*) Method B.
(*c*) Method B.

(3) What useful information, if any, did the pH of the flour give you?

Pearl Brown: "Should give some information regarding the extent of treatment or bleach."

S. M. Jackson: "None."

J. Micka: "The pH value may give some information about the bleach (to what extent) of the flour."

C. O. Oppen: "None excepting in the cake types."

Mr. Ulrey: "None."

Kroger Foundation: "Experience with the test limited; pH was capable of some correlation with degree of bleach, but this was not made use of at all in the classifications."

(4) Does the change in viscosity between the first and second readings tell you anything about—

(*a*) The quality of a flour?

(*b*) The buffer capacity of a flour?

S. M. Jackson: "(*a*) No.
"(*b*) To some extent."

J. Micka: "Viscosity on the whole is very helpful on short as well as long and straight patent flours. The shorter the extraction, the greater the variation between the first and second readings, provided the flours are made from the same wheat blend. Due to the buffer action, viscosity reading on cut straights and especially on clears and low grades is problematical, and I don't think this could be utilized for actual baking operations of the flour."

C. O. Oppen: "(*a*) We are able to predict the ash content on our patent grades within 0.01% by noting the change between the first and second reading.
"(*b*) It does that on the first two readings."

E. L. Ulrey: "(*a*) Yes.
"(*b*) Yes."

Kroger Foundation: "(*a*) Yes. Within given types of flour and in comparison with the other differences and the total reading. Under these conditions a greater difference between the first and second reading will accompany shorter extraction.
"(*b*) While this change cannot be taken as a precise or strictly proportional measure of buffer capacity, we interpret a smaller difference between first and second readings as indicating greater buffer capacity (this of course where maximum reading is the same).

The agreement between the ash results is quite satisfactory. The only significance of this determination is to indicate the approximate degree of extraction of the flour. The protein results reported by the collaborators on flours 1 and 2 come within satisfactory checking limits. The protein results for flour No. 3 show a somewhat greater variability. These figures establish the fact that flour No. 2 is approximately 0.60% lower in protein content than flour No. 1, while that of No. 3 is about 1.4% lower. The lack of agreement in the pH results is undoubtedly due to the fact that no standard method was used in this determination. Apparently within our present knowledge, judging by the answers to the questionnaire, no significance can be attached to this test other than establishing approximately the relative degree of bleaching or maturing treatment.

While the agreement between the viscosity results may not entirely measure up to expectations, there is sufficient concordance to offer encouragement for the future. Certainly it can be said that in using this factor for classifying the flours, the collaborators were able to arrive at a unanimous opinion regarding the relative strength of the three flours; namely, that flour No. 1 is strongest, flour No. 3 is weakest, and flour No. 2 is of intermediate strength with respect to the other two. In addition, making due allowance for the fact that apparently the tentative standard nomenclature proposed by Bohn (1935) is not generally used, there is very satisfactory agreement among the collaborators in classifying the flours studied in their various applications in the biscuit and cracker industry.

An examination of Tables IV, V, and VI shows satisfactory agreement (except for the figures on volume) between the various collaborators with the results of the baking test by either method A or B. The description of the loaves might even be closer if a more rigid system of descriptive terms were available. The rather wide discrepancy between the figures on volume may be attributed in part to the fact that all the collaborators did not use the same size of baking pan. There is also reason to believe that individual differences in molding and panning technique may contribute to this condition. Just as with the viscosity test, the collaborators ranked the three flours in the same order of relative strength and are in virtual agreement concerning their commercial application as determined by means of the baking test. A rather striking fact, however, is revealed by these data in that the collaborators ranked flours 1 and 2 a degree higher in strength by means of the baking test than they did by the viscosity results. This difference was not brought out in the previous work of this committee and may indicate that in the final analysis the baking test may be the more reliable means for classifying biscuit and cracker flours. The viscosity test, however, should not be discredited as a result of this difference, since it is known from general experience that flours 1 and 2 do exhibit unusual baking characteristics in the light of the viscosity values they yield. The need for more work and additional data along these lines is therefore apparent.

A comparison of the results obtained by baking methods A and B reveals that either method will satisfactorily classify biscuit and cracker flours. However, method B may show individual differences between flours a little more sharply than does method A, and may be more easily adapted to the routine of those laboratories where flours other than soft winter wheat types are tested as well.

The remarks listed in Table VII contribute additional evidence that operators in different laboratories can arrive at the same conclusions with regard to different flours by means of the baking test. This conclusion is warranted in that the various collaborators were able to take the baking results of another laboratory and arrive at the same conclusions about the flours as they did from their own baking tests, indicating thereby that despite seeming differences a sufficiently characteristic picture is obtained for each of the three types of biscuit and cracker flours.

Conclusions

As a result of the work done this year, it may be concluded:

That the baking test is a reliable means for classifying biscuit and cracker flours, and, in addition, offers the promise of yielding concordant results in the hands of different operators.

That confirmation is given to the earlier work done to the effect that the viscosity test reliably classifies flours in the order of their relative strength.

That the need for a greater background of experience and data in classifying biscuit and cracker flours by means of the baking and/or viscosity test is shown.

Recommendations

The Committee therefore recommends that:

Work be repeated on other flours, subjecting the viscosity and baking test to further examination employing essentially the technique herein outlined.

All other questions raised by the previous committee be tabled until a more comprehensive understanding has been arrived at with respect to fundamentals.

A start be made to establish a definite correlation between laboratory and actual shop results by means of carefully controlled laboratory tests and practical tests on a commercial basis.

Acknowledgments

This work would not have been possible without the cooperation of the members of this committee who were Miss Pearl Brown, C. O. Oppen, E. L. Ulrey, S. M. Jackson, Jan Micka, and J. C. Lankenau. Thanks are alsso due to L. A. Mennel, Jr., of the Mennel Milling Company, who supplied the flour used in this investigation. Appreciation is also expressed to the staff of the Cereal Laboratory of the Kroger Food Foundation for assistance in carrying out the work, and to George Garnatz for much help in planning the tests and in preparing this report.

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REPORT OF THE COMMITTEE ON TESTING RYE FLOUR

L. H. BAILEY, *Chairman*

Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture, Washington, D. C.

(Read at the Annual Meeting, June 1936)

The Committee on Testing Rye Flour is able to make only a progress report at this time. There are many complex problems still unsolved.

At the beginning of the year, last year's numerical score card was changed as follows: The number of points allowed for a perfect score for crumb color was changed from 10 to 15, and the number of points for perfect grain was reduced from 15 to 10.

The individual members of the committee secured their own samples of light, medium and dark rye flours, and baked them into breads in the proportion of one part of rye flour to one part of clear flour. The light rye doughs were proofed for 55 minutes, the medium ryes for 45 minutes, and the dark ryes for 35 minutes.

The same rye flours were also baked using the following proportions: 50% of light rye, 40% of medium rye, and 30% of dark rye, the balance being clear flour. The proofing periods for these proportions were not specified. The members of the committee were requested to make ash determinations of the various rye flours and observe if there seemed to be a correlation between ash content and baking quality of the rye flours, and if the ash determination could be substituted for the baking test in determining the baking value; also to note if there was a correlation between flavor (odor and taste) and the color of the bread.

In general, the members of the committee are in agreement that an ash determination of the rye flour cannot be substituted for the baking test, although there is some correlation between the ash content and baking value, especially with the light and medium rye flours; also that there is a relationship between color and flavor especially where there are wide differences in color.

The proportions of 50% light rye, 40% medium rye, and 30% dark rye are preferred to using 50% of all grades of rye. Four samples of medium rye flour were furnished the members of the committee for collaborative baking tests. Instructions were given to use 40% rye flour and 60% of the collaborator's clear flour. The doughs were to be mixed for 2 minutes on the Hobart-Swanson mixer, using 2% yeast and 2% salt, fermented for 3 hours, and proofed for 45 minutes.

The baking results are shown in Table I. The collaborators are not all in agreement as to the baking qualities of these four flours, two of which were average medium rye flours and the other two were dark-medium rye flours.

TABLE I

COLLABORATORS' SCORES OF RYE BREADS

Sample	Weight	Volume	General appearance 25	Crust color 10	Crumb color 15	Grain 10	Texture 15	Odor and taste 25	Total score
	Grams	*Cc.*	*Score*	*Score*	*Score*	*Score*	*Score*	*Score*	
				First Collaborator					
A	271	830	23	10	15	8	13	21	90
B	272	755	23	8 D	7 Dk	9	14	23	84 Dark
C	269	870	23	10	15	8	13	21	90
D	268	780	23	8 D	9 Dk	9	14	23	86 Dark
				Second Collaborator					
A	285	798	24	9	13	8	14	23	91
B	300	666	24	8	15	9	14	25	95
C	285	782	23	9	13	8	13	23	89
D	300	697	23	9	15	8	13	24	92
				Third Collaborator					
A	315	1130[1]	24	10	14	8	13	24	93
B	314	1032	22	10	12	9	11	23	87
C	313	1114	23	9	13	9	12	22	88
D	311	1327	25	10	15	7	14	20	91
				Fourth Collaborator					
A	275	820	22	10	14	9	14	25	94
B	285	680	23	8	8	10	15	25	89
C	275	855	23	10	14	9	14	25	95
D	280	675	21	8	10	10	15	25	89
				Fifth Collaborator					
A	284	835	21	9	13	9	13	25	90.0
B	295	705	20	8	9	7.5	12	25	81.5
C	279	870	22	8.5	14	9	12	25	90.5
D	292	770	19	8.5	10	6.5	11	25	80.0
				Average Results					
A	286	821	23	9.6	14	8	13	24	
B	293	702	22	8.4	10	9	13	24	
C	284	844	23	9.3	14	9	13	23	
D	290	730	22	8.7	12	8	13	23	

[1] Omitted from average.

It has been suggested to reduce the mixing time on the Hobart-Swanson mixer to 1 minute and to proof to the optimum, rather than for a definite period.

The collaborators have experienced difficulty in arriving at a conclusion as to the way that the color of the crumb should be scored. The committee is considering the feasibility of developing a series of color cards to match the colors of different rye breads and then score crumb color according to a color card in the series and not according to any numerical value. In such scoring, one color would not be given

preference over another, but it would have a definite designation just as we now designate a loaf of white bread as Type F or grain as No. 5.

Recommendations

It is recommended:

That the work on testing rye flour be continued.

That the next committee give consideration to the development of a series of colors that may be used as standards for matching the color of rye bread crumb of the different grades of rye flour.

REPORT OF THE COMMITTEE ON METHODS OF ANALYSIS

B. SULLIVAN, *Chairman*

Russell-Miller Milling Company, Minneapolis, Minnesota

(Read at the Annual Meeting, June 1936)

General Report

Subjects which have been studied this year by members of the Methods Committee include the following: the effect of different types of shortening on white pan bread; the evaluation of the effect of milk solids in dough fermentation; the influence of individual experimental milling technique on loaf characteristics; a collaborative comparison of methods for estimating the diastatic activity of flour. Viscosity measurements and their significance have been investigated by the Subcommittee on Viscosity.

As can be seen from the above titles, the Methods Committee this year has endeavored to enlarge the scope of its work. In addition to the testing of various new procedures and the critical analysis of certain methods, it has been deemed advisable to study the evaluation of materials other than flour which are used in breadmaking.

Recommendations

It is recommended:

That more extended research be done relative to the evaluation of shortening, milk, malt, yeast, yeast foods, and other ingredients which are used in breadmaking.

That the determination of the moisture content of wheat by means of the Tag-Heppenstall moisture meter be investigated.

That the subject of laboratory experimental milling be further studied and an effort made to approach more uniform conditions in laboratory milling procedures.

That methods for the measurement of the proteolytic activity of flour be investigated.

REPORT OF THE SUBCOMMITTEE ON VISCOSITY [1]

E. G. BAYFIELD,[2] *Chairman*

Ohio Agricultural Experiment Station, Wooster, Ohio

(Read at the Annual Meeting, June 1936)

Upon completion of the present year's work, this subcommittee rounds out four years of activity, and believes that some progress may now be considered as having been made. Aside from the increasing faith of this committee in the viscosity test as a result of its members using the test in everyday work, the findings of other A. A. C. C. committees testing soft wheat flour products are becoming increasingly favorable. The Committee on Testing Biscuit and Cracker Flours (Bohn, 1935) has been able to set tentative viscosity standards for some cracker and biscuit flours. Reporting for the committee, Bohn states, "An examination of the viscosity results compared to the commercial utility of the flours shows that viscosity bears a definite relation to strength as determined by shop practice." The Pie Flour Committee (Kress, 1936), after studying results of various tests employed, states, "The *viscosity* test apparently gives the best information of any single test." Somewhat modified viscosity procedures from that suggested as a tentative method in Cereal Chemistry **10**: 494–501 (1933), were employed by the committees directed by Bohn (1935) and Kress (1936). The use of these variations in procedure has plagued this Subcommittee on Viscosity since its inception. It is satisfying to know, however, that gradually the various users of the test are beginning to adopt *one* procedure. It is to be hoped that the present report may further aid in the adoption of a uniform viscosity technique.

The present report covers work which may, for convenience, be divided into two phases: the first being results of experimental testing of possible procedures by the chairman, and, the second consisting of collaborative test work on the part of various members of the subcommittee acting as a group.

Experimental Studies

STABILITY OF LACTIC ACID SOLUTIONS.—In April 1934 several liters of lactic acid were made according to the method of Reiman (1934).

[1] Subcommittee report, 1935–36 Committee on Methods of Analysis.
[2] Associate in Agronomy and Cereal Chemist, Ohio Agricultural Experiment Station.

This solution has occasionally been checked for normality. At the end of two years the solution was at essentially the same normality, indicating that a stock supply may be kept for at least this length of time without any significant change in normality.

Effect of Added Starch upon Flour Suspension.—Bayfield (1934, 1935), in attempting to eliminate possible variations in results due to the differences in bulk of flour which arise when viscosities are determined upon a flour sample containing 2.0 g. of protein (constant protein method), obtained inconclusive results which were thought to be due to the type of starch added in the tests.

To collect further data as to the possibilities of developing the constant protein method for testing the quality of protein along these lines, four commercial starches were obtained. Starches 2 and 3 were high-grade starches of the so-called "untreated" type, whereas starch 4 was a treated "thin boiling" starch. Starch 1 was specially prepared for the author through the courtesy of L. M. Thomas of the Keever Starch Company of Columbus, Ohio. This special batch was prepared in the following manner (personal communication from L. M. Thomas, 1936):

"It was washed from the flour with our well water, passed through No. 20 XX silk bolting cloth while in suspension and then allowed to settle out on the starch tables. The settled starch was taken up 5 hours after the start of the day's run, again suspended in water and screened once more. The water was then partly removed by filtering in a muslin bag filter, fermentation being prevented by keeping the temperature below 45 degrees F. The resulting solid cake of starch was then dried in a kiln at not to exceed 140° F. The drying time was 24 hours."

It will be seen therefore that starch 1 was not treated with chemicals in any way and might be expected to act like natural wheat starch. Analytical data obtained on these starches are given in Table I. It

TABLE I

Analytical Data Obtained on Four Starch Samples

Sample number	Crude protein	Ash	Moisture	Viscosity—30 g. sample, 100 cc. water				
				No acid	1 cc. acid	3 cc. acid	5 cc. acid	7 cc. acid
	%	%	%	°*MacM.*	°*MacM.*	°*MacM.*	°*MacM.*	°*MacM.*
1	0.28	0.236	6.2	2+	3+	3	3	2+
2	0.48	0.231	6.4	4+	9−	9	9	8+
3	0.36	0.160	6.5	2+	4+	4+	4	3+
4	0.36	0.370	6.4	2−	4+	3+	4−	3+

may be observed, excepting starch 2, that even a 30 g. sample of starch possesses a low viscosity. Ten grams of starch 1 when suspended in 100 cc. of distilled water, gave readings as follows: — 1, 1, 0.5, 0 +, and 0° MacM. for the increasing amounts of acid, respectively. Increasing the amount of starch 1 to 15 g. in 100 cc. water gave a maximum reading of less than 2° MacM. (with 1 cc. of N lactic acid).

To compare the effect of the four starches upon viscosity when added to flour, varying amounts of each starch were added to 20 g. (not corrected to 15% moisture) of soft winter wheat flour and suspended by shaking for 1 minute (method details given later) and the viscosity determined as in the usual no-time method. The results obtained with this flour (Flour A) are given in Table II.

TABLE II

EFFECT OF ADDED STARCH UPON VISCOSITY OF FLOUR A

Acid used	Starch used	Viscosity in degrees MacMichael													
		None	1				2			3			4		
	Starch added, grams	0	5	10	20	40	5	10	20	5	10	20	5	10	20
Cc.															
0		19	20	21	26	43	27	39	67	21	24	32	19	20	27
1		34	35	35	40	71	36	38	53	37	40	53	27	23	32
3		71	75	79	88	138	82	89	121	77	83	105	67	66	68
5		78	82	86	96	146	91	99	144	85	92	112	77	77	83
7		80	83	86	95	140	91	101	144	85	92	108	79	81	87

Examination of these data show several interesting points:

(*a*) No two of the starch samples produced the same results when added to a given amount of flour A.

(*b*) Starch 1 resembled most closely the starch B used by Bayfield (1934) in an earlier investigation. However, the resemblance in results is not close.

(*c*) Starch 2, a so-called "untreated" high grade edible starch, was treated with a 0.2% sulphurous acid solution to prevent fermentation during manufacturing. It gave results consistently higher than the others.

(*d*) Less is known about the exact manufacturing procedure used with starch 3, but it is a high grade "thick-boiling" starch. Results with this starch lay between starches 1 and 2.

(*e*) Starch 4, a "thin-boiling," treated starch, gave results quite different from the other three samples in that it reduced the viscosity of the acidulated flour-water suspension.

(*f*) Starch 1 appeared to be the most promising for test purposes in creating additional bulk in the sample when using the constant protein method.

USE OF STARCH 1 WITH EXPERIMENTALLY MILLED VARIETY SAMPLES.—It so happened that environmental conditions in 1934 influenced a series of varieties in such a way that high protein wheat resulted at one location whereas low protein samples resulted at the other. Four of these varieties were selected for preliminary testing with starch 1. Sufficient of this starch was added to the high protein samples so that the total weight of starch plus flour equaled the weight of the 2-g. low protein samples. Results are presented in Table III. The results ob-

TABLE III

EFFECT OF ADDED STARCH UPON VISCOSITY[1] OF HIGH PROTEIN SAMPLES

Variety	American Banner			Gladden			Fulhio			Michikof		
Sample number	2526	2446		2531	2451		2524	2444		2533	2460	
Flour protein, %	8.2	13.3		9.0	12.5		9.6	13.3		9.7	15.1	
Flour ash, %	0.35	0.36		0.36	0.39		0.33	0.38		0.42	0.41	
Weight flour used, g.	23.8	14.6	14.6	21.8	15.6	15.6	20.4	14.6	14.6	20.0	12.9	12.9
Starch added, g	0.0	0.0	9.2	0.0	0.0	6.2	0.0	0.0	5.8	0.0	0.0	7.1
Viscosity, ° *Mac M.*, no acid	13	12	11	17	9	8	13	7	7	18	8	7
Viscosity, ° *MacM.*, 1 cc. acid	59	78	68	75	54	42	103	66	55	116	77	60
Viscosity, ° *MacM.*, 3 cc. acid	102	109	100	115	82	68	149	97	88	197	133	105
Viscosity, ° *MacM.*, 5 cc. acid	110	113	106	119	88	74	152	105	94	217	143	121
Viscosity, ° *MacM.*, 7 cc. acid	111	112	107	120	88	76	153	105	96	222	146	129

[1] Viscosities obtained according to method employed by Bayfield (1935, page 4).

tained from the use of added starch with these variety samples seem to indicate that further work along these lines is unjustified at this time. Examination of the data indicates quite plainly that protein diluted by starch gives entirely different results from the same amount of protein when naturally distributed throughout starch during formation of the wheat berry. This is not a particularly unexpected conclusion when the colloidal nature of flour is considered.

METHODS FOR MAKING FLOUR-WATER SUSPENSIONS.—The use of a mortar and pestle is stipulated for making the flour-water suspension in the tentative procedure outlined earlier by this subcommittee (Bayfield, 1933). This method has been found to possess a number of disadvantages, principally those due to variations resulting from the operator using the pestle. Mechanical mixers operated by electric motors would prove satisfactory provided all laboratories were equipped with identical equipment. Preliminary trials with electric mixers as found in the various laboratories, however, indicated that the use of these machines would introduce more variations in results than obtained by the use of the mortar and pestle.

As a result of these various efforts an excellent idea was advanced by George Alexander who suggested shaking the flour with the desired

amount of water in an Erlenmeyer flask. Preliminary trials with this method by the chairman and several members of the subcommittee proved so satisfactory that it was decided to use it as one procedure in the collaborative work to be described later. Smoother suspensions resulted, and variations in gluten development due to the operator should be largely eliminated by this method.

To determine the optimum time required for shaking, a considerable number of tests were made by the writer, using a range in flours. As might be expected no trouble was experienced in obtaining a smooth suspension with granular flours. Even with starchy-type flours, inclined to be lumpy, about a minute of shaking gave smoother suspensions than those obtained by the use of the mortar and pestle. The degree of lumpiness of these suspensions was noted by pouring the suspensions through a wire gauze screen.

A series of samples of flours A and B were then run to determine what effect was produced by varying the time of shaking. In these shaking experiments shaking was done by hand with 180 to 200 shakes being given per minute. Both "no-time" (no digestion) and "one-hour digestion" methods were used. Duplicate determinations gave satisfactory checks in all cases excepting with the shortest (¾ minute) shaking period used. Three-quarters of a minute produced somewhat lumpy suspensions. The results are presented in Table IV. The mortar and pestle were employed for making comparative suspensions.

TABLE IV

EFFECT UPON VISCOSITY OF TIME OF SHAKING THE SUSPENSIONS

Time of shaking, minutes	Flour A						Flour B				
	0*	¾	1.0	1½	2.0	5.0	0*	¾	1.0	1½	2.0
	No digestion period										
Viscosity, ° *MacM.*, no acid	11	17	16	15	14	13	13	21	22	21	21
Viscosity, ° *MacM.*, 1 cc. acid	28	32	32	32	31	31	88	102	103	101	101
Viscosity, ° *MacM.*, 3 cc. acid	56	59	60	60	60	61	226	218	220	214	215
Viscosity, ° *MacM.*, 5 cc. acid	61	64	66	65	66	67	254	240	238	237	239
Viscosity, ° *MacM.*, 7 cc. acid	63	66	67	67	67	68	263	244	243	241	244
Viscosity, ° *MacM.*, 9 cc. acid	62	66	—	64	63	65	260	242	242	238	240
	Shaking followed by 1 hour digestion period at 30° C.										
Viscosity, ° *MacM.*, no acid	9*	9	9	8	8		14*	20	20	19	19
Viscosity, ° *MacM.*, 1 cc. acid	50	50	50	49	48		153	167	162	151	152
Viscosity, ° *MacM.*, 3 cc. acid	72	71	70	69	70	Not	306	308	291	277	275
Viscosity, ° *MacM.*, 5 cc. acid	77	73	73	73	73	run	345	333	323	303	305
Viscosity, ° *MacM.*, 7 cc. acid	78	74	74	73	74		360	342	332	314	314
Viscosity, ° *MacM.*, 9 cc. acid	77	71	72	73	73		363	342	331	311	314

* Mortar and pestle method.

The data in Table IV indicate that suspensions prepared by shaking in an Erlenmeyer flask do not give viscosity results the same as those prepared by means of a mortar and pestle. Judging by the appearance of the suspensions, it appeared as though the mortar and pestle method with a 55-second working period gave as many lumps as a ¾-minute shaking period. A 1-minute shaking period gave suspensions practically free from lumps and is to be recommended, seeing that no benefit results from prolonging the shaking for a longer time.

In the above shaking tests the weighed amount of flour was placed in a dry 500 cc. Erlenmeyer flask, 100 cc. of distilled water at 30° C. quickly poured in, the flask stoppered, and immediately shaken vigorously for the required interval. The resulting suspension was poured into the viscosimeter bowl, caprylic alcohol added, and the viscosity determination then made in the usual manner. Where a 1-hour digestion period was used, the flask containing the suspension was held in an air bath at 30° C. before pouring into the bowl of the viscosimeter.

It was later found by placing 3 to 4 drops of caprylic alcohol in the flask immediately after shaking, re-stoppering, and then giving 10 additional shakes to the flask, that the foam produced during shaking was handled to better advantage than by placing the caprylic alcohol in the viscosimeter bowl. A cleaner pour also resulted. However, no large differences in the viscosity readings resulted from this variation in time of adding the caprylic alcohol. It was further found that the small amount of flour which remains in the Erlenmeyer flask after pouring out the suspension produces no significant effect upon the viscosity reading and may thus be disregarded for practical purposes.

Collaborative Studies

These studies may be divided into two parts: (*a*) results of testing two flours sent out by the chairman, by each of three methods, and (*b*) results obtained by the same three methods when employed upon two flours familiar to the individual collaborators. The purpose of these latter tests was to give each collaborator an opportunity to test each of the three methods upon flours, the characteristics and utilities of which he was quite familiar with. Each collaborator provided, in addition to viscosity data, the protein, moisture, and ash analyses, as well as defining the type of flour which he used as his selected flours. All flours were regular commercial flours.

In forwarding directions for collaborative testing of the samples, all collaborators were requested to observe as closely as possible the two following precautions:

TABLE V

Collaborative Results Obtained on Flour A, a Soft Winter Wheat Cake Flour

(All results in degrees MacMichael)

Collaborator	A			B			C			D			E			F			G			H			I			J		Range			Mean
Acid, *cc.*	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Min.	Max.	Diff.	
Method (*a*)—Mortar and pestle																																	
0	11	11	11	10	9	10	8	9	9	12	11	12	10	10	10	9	9	9	10	9	10	8	9	9	7	8	8	8	8	7	12	5	9
1	28	28	28	27	33	30	34	33	34	40	40	40	39	38	39	49	47	48	33	34	34	32	31	32	30	29	30	28	29	27	49	22	34
3	56	55	56	54	57	56	66	64	65	62	61	62	67	67	67	68	70	69	63	59	61	56	55	56	55	53	54	52	54	52	70	18	60
5	61	61	61	59	62	61	73	72	73	65	65	65	74	74	74	69	72	71	68	65	67	63	61	62	60	58	59	58	60	58	74	16	65
7	63	62	63	62	64	63	73	73	73	66	66	66	74	74	74	69	72	71	68	66	67	66	64	65	61	58	60	62	61	58	74	16	66
9	62	—	62	62	64	63	73	72	73	65	64	65	74	74	74	68	71	70	—	—	—	63	62	63	61	58	60	—	—	—	—	—	66
Method (*b*)—1 minute shaking, no digestion																																	
0	16	16	16	13	13	13	15	15	15	17	17	17	13	15	14	10	12	11	15	15	15	13	15	14	8	9	9	10	10	8	17	9	13
1	32	32	32	30	31	31	34	35	35	37	42	40	40	41	41	38	40	39	34	34	34	31	30	31	29	30	30	38	35	29	42	13	35
3	60	60	60	59	60	60	67	67	67	59	63	61	66	67	67	59	63	61	60	60	60	57	57	57	53	55	54	58	62	53	67	14	61
5	65	66	66	64	64	64	74	73	74	64	70	67	73	74	74	63	65	64	66	65	66	63	62	63	58	60	59	66	69	58	74	16	66
7	66	68	67	64	64	64	75	74	75	66	68	67	73	74	74	64	67	66	67	67	67	64	63	64	60	62	61	69	70	60	75	15	67
9	—	—	—	64	63	64	74	73	74	65	65	65	73	74	74	63	65	64	—	—	—	61	60	61	60	64	62	—	—	—	—	—	66
Method (*c*)—1 minute shaking, 1 hour digestion																																	
0	9	8	9	6	7	7	9	9	9	12	11	12	11	12	12	8	8	8	9	9	9	16	13	15	8	8	8	10	10	6	16	10	10
1	50	50	50	51	50	51	55	54	55	53	51	52	61	62	62	55	55	55	50	51	51	45	44	45	39	42	41	43	46	39	62	23	50
3	70	70	70	70	71	71	76	76	76	71	71	71	85	86	86	80	78	79	73	74	74	63	63	63	63	67	65	63	69	63	86	23	72
5	73	73	73	76	76	76	81	81	81	74	75	75	87	89	88	85	83	84	76	77	77	66	67	67	68	70	69	73	74	66	89	23	76
7	73	74	74	77	79	78	81	82	82	74	75	75	87	89	88	83	82	83	76	77	77	67	68	68	63	70	69	73	75	67	89	22	77
9	72	—	72	77	77	77	80	81	81	72	73	73	87	89	88	80	78	79	—	—	—	60	60	63	68	70	67	—	—	—	—	—	75

TABLE VI

Collaborative Results Obtained on Flour B, a Strong Bread Flour

(All results in degrees MacMichael)

Collaborator	A			B			C			D			E			F			G			H			I			J		Range			Mean
Acid, *cc.*	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Min.	Max.	Diff.	
Method (*a*)—Mortar and pestle																																	
0	12	13	13	15	17	16	15	14	15	18	17	18	16	16	16	23	23	23	13	13	13	12	14	13	15	15	15	12	12	12	23	11	15
1	88	88	88	85	75	80	109	110	110	128	125	127	111	112	112	117	120	119	102	100	101	108	110	109	100	90	95	85	89	75	128	53	103
3	228	224	226	206	185	196	250	255	253	265	250	258	258	250	254	264	262	263	233	234	234	236	240	238	239	247	243	197	225	185	265	80	237
5	252	256	254	227	260	244	280	285	283	289	285	287	282	279	281	280	285	283	267	264	266	256	260	258	275	295	285	230	253	227	295	68	268
7	264	262	263	250	250	250	292	298	295	293	290	292	293	288	291	285	290	288	272	271	272	262	265	264	283	282	283	237	253	237	298	61	274
9	260	—	—	240	243	242	291	298	295	290	285	288	293	288	291	290	290	290	268	270	269	255	260	258	285	282	284	—	—	—	—	—	276
Method (*b*)—1 minute shaking, no digestion																																	
0	22	22	22	22	23	23	17	17	17	22	21	22	19	17	18	20	20	20	20	20	20	19	18	19	16	15	16	15	15	15	23	8	19
1	103	102	103	110	115	113	106	107	107	130	115	123	125	123	124	115	117	116	99	97	98	98	100	99	100	105	103	95	100	95	130	35	108
3	221	218	220	200	205	203	231	223	227	230	229	230	248	250	249	210	212	211	213	213	213	215	220	218	248	235	242	202	204	200	250	50	221
5	237	239	238	220	227	224	255	251	253	254	254	254	278	275	277	230	234	232	238	234	236	246	250	248	282	268	275	230	229	220	282	62	247
7	242	244	243	225	230	228	261	260	261	263	262	263	285	284	285	230	234	232	241	240	241	255	259	257	285	270	278	237	235	225	285	60	252
9	243	240	242	223	229	226	262	258	260	254	258	256	285	284	285	225	230	228	238	240	239	243	250	247	285	270	278	—	—	—	—	—	251
Method (*c*)—1 minute shaking, 1 hour digestion																																	
0	19	20	20	20	19	20	16	17	17	20	19	20	23	23	23	20	20	20	18	18	18	21	20	21	16	15	16	20	19	15	23	8	19
1	162	161	162	190	142	166	166	176	171	180	178	179	188	187	188	180	182	181	167	164	166	160	155	158	138	140	139	151	167	138	190	52	167
3	290	292	291	290	275	283	292	304	298	299	300	300	311	313	312	330	315	323	300	300	300	272	270	271	260	280	270	275	265	260	330	70	292
5	323	322	323	320	302	311	324	334	329	327	328	328	340	344	342	345	335	340	330	327	329	302	305	304	298	309	304	304	304	298	345	47	321
7	334	329	332	320	312	316	329	343	336	337	334	336	346	349	348	350	340	345	340	339	340	312	314	313	305	316	311	306	305	305	349	44	328
9	332	330	331	311	312	312	327	344	336	340	338	339	346	349	348	345	335	340	338	336	337	312	312	312	314	316	315	—	—	—	—	—	330

1. All tests were to be made at 30° C. It has been found desirable to have water used, equipment, etc., as close to the correct temperature as possible.

2. Viscosimeters were to be so set that there was a disk clearance, *i.e.*, the distance between lower surface of bob and the bottom of the bowl, of 0.250 ± .005 inches. It has been decided to standardize upon this figure as a result of the work of the A. O. A. C. Viscosity Committee under the direction of Associate Referee C. G. Harrel (1935). The disk clearance is to be measured with a micrometer depth gauge. (This did not prove possible in the case of all collaborators reporting results in these studies.)

The three procedures to be employed on the flours were as follows:

(*a*) Observing the above two precautions, make the determinations using the no-time several-increment method outlined in Cereal Chemistry **10:** 499 (Bayfield, 1933).

(*b*) Substitute for the mortar and pestle used in (*a*) the following method for making the suspension, otherwise the procedure is the same as in (*a*). Place 20 g. flour (15% moisture basis) in a 500 cc. *dry* Erlenmeyer flask, add 100 cc. of distilled water, previously warmed to 30° C. Shake vigorously for 1 minute after tightly stoppering the flask. Remove stopper and pour into viscosimeter bowl, add 3 to 4 drops caprylic alcohol, stir with bob 25 times and then make viscosity determinations.

(*c*) Repeat as in (*b*) excepting that after making suspension, the flask is placed in a water or air bath held at 30° C. and the suspension is allowed to digest for 1 hour at this temperature. The flask is rotated a few times at approximately 30 minutes digestion and again at the end of the digestion period to aid in keeping the flour in suspension. (It will be observed that this method differs slightly from that recommended later on page 785.)

Collaborative Results from Flours Tested by All Collaborators.—The two flours tested by each collaborator were of widely different character. Flour A was an all soft red winter cake flour, ash 0.37 %, and protein 8.40%. Flour B was a granular all spring bread flour, ash 0.37%, and protein 13.06%. This last flour was included in the test as an example of a more difficult type of flour upon which to obtain satisfactory checks. Any method proving satisfactory with such strong samples should easily handle the weaker soft wheat flours.

Reports of 10 collaborators are available, the results for flour A being given in Table V while those for flour B may be found in Table VI. In these two tables individual readings are presented as well as the average for the two determinations made by each operator. The results obtained by collaborator J, however, have not been averaged as the two single determinations given were obtained by two operators and several days elapsed between the first determination and the duplicate. The last column "Mean" gives the average for all 20 determinations made upon the flour.

Examination of the collaborative results indicates several points:

(*a*) Some collaborators obtained consistently higher results with all methods (*e.g.*, collaborators C and E) indicating that some uncontrolled factor was operative in these laboratories. Others obtained consistently low results (collaborator J with flour B). No explanation as to the reason for these differences is available.

(*b*) Duplicate determinations in some instances varied so widely that

they could hardly be described as being even "reasonable checks." This condition applied particularly in the case of flour B and indicates a lack of experience in handling strong flours in so far as some collaborators are concerned. Several of the collaborators had previous occasion to handle flours of the type of flour B and their duplicate determinations were in much closer agreement.

(*c*) Most of the collaborators possessed greater familiarity with method (*a*) than with either of the other two procedures under test. This fact should influence the results in favor of method (*a*) and practically prohibits any adequate statistical comparison of the data accumulated by the three methods. Such a statistical examination would be very desirable provided the data justified its use.

(*d*) While the variation in results is unaccountably large, yet several of the collaborators obtained data which agreed reasonably well. These data indicate that it is possible for laboratories to check each other's results provided controlled conditions hold. The empirical nature of the viscosity determination must again be emphasized.

(*e*) Some idea as to the variation existing in the data may be noted by examining the "'range" columns. It will also be noted with both flours A and B that a smaller average difference occurs between the minimum and maximum figures with method (*b*) than with method (*a*). This indicates a closer agreement in results with method (*b*) than with the other no-time procedure, *i.e.*, method (*a*). The nature of method (*c*) gives a wider spread in results and therefore it may not be fair to the method to compare it directly in this manner with methods (*a*) and (*b*). However, such a comparison of the difference in range for flour B shows that with this strong flour method (*c*) is giving a smaller degree of variability than method (*a*). With flour A, however, method (*a*) gives less variation than method (*c*).

Collaborators' Results with Various Types of Flours.—The results for these tests (Table VII) differ from those just discussed in that all the collaborators worked with flours the approximate strengths of which they were familiar with. As a result the data are more favorable. With method (*a*) only in one case (collaborator B, cracker flour) did the checks differ by more than 4° MacM. In no case did method (*b*) give duplicate determinations differing by more than 4° MacM. Method (*c*) does not show as good a record as method (*b*), as collaborators A (bread), E (bread), I (cracker), and B (cracker, and cookie) show duplicate readings which did not check within 4° MacM. However, with this method the maximum viscosity reading in only one instance shows duplicate determinations which did not check within 4° MacM. In this case collaborator A gives duplicates showing a 5° variation in maximum viscosity for a strong bread flour.

TABLE VII

Viscosity Data Obtained by 9 Collaborators Using Various Types of Flours

(All data in degrees MacMichael)

Collaborator	A			G			F			E			H			I			B			D			C			A		
Flour type	Bread			Bread			Bread			Bread			Family			Cracker			Cracker			Cracker			Cracker			Cake		
Acid, *cc.*	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.
Method (*a*)—Mortar and pestle																														
0	12	13	13	12	12	12	15	15	15	10	10	10	8	10	9	8	8	8	13	12	13	11	10	11	7	7	7	11	11	11
1	88	88	88	91	90	91	50	50	50	67	68	68	34	35	35	36	38	37	30	34	32	40	39	40	34	34	34	28	28	28
3	228	224	226	189	188	189	130	132	131	114	114	114	78	80	79	55	58	57	58	51	55	60	60	60	55	57	56	56	55	56
5	252	256	254	213	210	212	140	140	140	125	126	126	93	96	95	68	68	68	61	57	59	64	64	64	60	61	61	61	61	61
7	264	262	263	218	215	217	145	145	145	126	126	126	96	97	97	68	68	68	67	62	65	65	65	65	60	62	61	63	62	63
9	260	—	—	218	215	217	150	145	148	126	126	126	95	95	95	69	69	69	67	62	65	63	64	64	59	60	60	62	—	—
Method (*b*)—1 minute shaking, no digestion																														
0	22	22	22	17	19	18	15	15	15	14	14	14	13	11	12	12	12	12	16	17	17	15	14	15	10	10	10	16	16	16
1	103	102	103	89	91	90	50	50	50	85	85	85	35	36	36	38	34	36	44	43	44	39	39	39	36	33	35	32	32	32
3	221	218	220	175	178	177	120	118	119	127	130	129	78	80	79	70	67	69	65	66	66	60	61	61	57	54	56	60	60	60
5	237	239	238	195	194	195	135	131	133	134	136	135	93	96	95	72	70	71	71	71	71	63	64	64	61	58	60	65	66	66
7	242	244	243	198	198	198	140	138	139	134	136	135	95	97	96	72	70	71	70	69	70	63	64	64	61	58	60	66	68	67
9	243	240	242	198	200	199	135	133	134	134	136	135	93	94	94	72	70	71	68	68	68	61	62	62	60	56	58	—	—	—
Method (*c*)—1 minute shaking, 1 hour digestion																														
0	19	20	20	15	15	15	15	17	16	12	12	12	12	13	13	8	8	8	8	7	8	10	10	10	7	7	7	9	8	9
1	162	161	162	144	146	145	97	95	96	97	101	99	65	68	67	42	44	43	60	55	58	52	52	52	47	48	48	50	50	50
3	290	292	291	251	253	252	175	173	174	144	156	150	113	115	114	64	67	66	85	79	82	73	73	73	66	69	68	70	70	70
5	323	322	323	277	279	278	190	190	190	164	166	165	120	122	121	76	70	73	90	85	88	78	77	78	71	72	72	73	73	73
7	334	329	332	283	284	284	193	191	192	165	168	167	127	129	128	76	77	77	90	87	89	78	77	78	70	74	72	73	74	74
9	332	330	331	284	285	285	185	185	185	165	168	167	124	125	125	76	77	77	89	87	88	76	76	76	69	72	71	72	—	—

TABLE VII—*Continued*

Collaborator	E			F			D			I			H			G			B			C			
Flour type	Cake			Cake			Cake			Cake			Cake			Pastry			Cookie			Cookie			
Acid, *cc.*	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	1	2	Av.	Mean
Method (*a*)—Mortar and pestle																									
0	7	7	7	8	8	8	8	8	8	5	6	6	8	10	9	6	7	7	10	10	10	5	4	5	9.2
1	39	39	39	38	38	38	30	33	32	27	26	27	20	21	21	9	9	9	26	22	24	17	17	17	39.3
3	52	52	52	52	52	52	40	42	41	32	33	33	28	29	29	24	23	24	35	34	35	23	22	23	72.6
5	54	54	54	53	53	53	44	45	45	32	33	33	31	32	32	29	29	29	39	38	39	24	24	24	80.3
7	54	54	54	53	53	53	44	45	45	32	33	33	28	29	29	31	31	31	40	38	39	24	24	24	81.9
9	54	54	54	51	51	51	43	45	44	32	33	33	29	28	29	31	31	31	40	39	40	23	23	23	—
Method (*b*)—1 minute shaking, no digestion																									
0	11	12	12	12	10	11	10	9	10	4	5	5	13	12	13	8	7	8	18	16	17	8	10	9	12.9
1	42	42	42	31	33	32	37	37	37	28	25	27	20	21	21	9	9	9	29	27	28	17	16	17	42.2
3	54	53	54	45	45	45	45	45	45	34	30	32	26	28	27	23	24	24	44	43	44	22	21	22	73.5
5	55	54	55	47	48	48	48	47	48	35	32	34	29	31	30	28	28	28	46	45	46	22	22	22	79.7
7	55	54	55	48	50	49	48	46	47	35	32	34	27	27	27	30	31	31	46	45	46	22	22	22	80.6
9	55	54	55	48	48	48	46	45	46	35	32	34	26	25	26	30	30	30	45	44	45	22	21	22	—
Method (*c*)—1 minute shaking, 1 hour digestion																									
0	7	7	7	5	5	5	8	7	8	5	6	6	9	10	10	6	5	6	5	5	5	5	4	5	9.2
1	41	43	42	38	40	39	34	37	36	32	30	31	20	21	21	23	22	23	30	35	33	19	19	19	58.9
3	55	58	57	55	55	55	49	50	50	35	32	34	27	28	28	45	45	45	50	54	52	26	25	26	93.5
5	59	63	61	57	57	57	53	55	54	36	36	36	30	30	30	49	50	50	58	59	59	28	27	28	101.8
7	63	66	65	60	60	60	56	57	57	36	37	37	31	30	31	50	50	50	57	60	59	29	28	29	104.1
9	63	66	65	58	58	58	54	56	55	36	37	37	30	30	30	49	50	50	57	61	59	28	27	28	—

Discussion of Collaborative Results.—In Tables V and VI it will be observed that on the average the strong flour B gives higher viscosity readings with method (*a*) than with method (*b*) whereas flour A gives higher results with method (*b*). In Table VII may be seen a tendency for method (*b*) to give higher results than with method (*a*) with the weaker flours while the opposite holds for the stronger flours. It seems reasonable to conclude that this effect is the result of variations in gluten development when the mortar and pestle is used for making the suspensions. Greater opportunity for gluten development exists with the strong flours with a high protein content and an increase in viscosity with method (*a*) will result. Furthermore, different operators will obtain varying amounts of gluten development and this probably accounts for much of the variability to be seen in Table VI with method (*a*).

It seems apparent, therefore, that method (*b*) is to be preferred over method (*a*) when these two no-time procedures are considered. Method (*b*) produces a smoother and more uniform suspension and less variation in gluten development occurs. The length of time required in making the viscosity determination is essentially the same in either case.

Method (*c*) possesses all the advantages of method (*b*) in so far as gluten development and smoothness of suspension are concerned. It has a further advantage in that it gives a wider spread in the magnitude of results. It therefore should be more sensitive to smaller differences in flour strength than method (*b*). Furthermore the 1-hour digestion period called for in method (*c*) will reduce variability in results due to variations in granulation existing in various flour samples as has been pointed out earlier by Bayfield (1934). The method will prove satisfactory for research purposes with experimentally milled flours. Bayfield (1935) found that no-time procedures were less satisfactory than a method employing a digestion period when studying such experimentally milled flours.

The chief disadvantage to method (*c*) is that it is not so well adapted to routine laboratory control purposes on account of the extra time required for the digestion period. Furthermore, differences in granulation are important in many soft wheat flours and these differences are largely masked by employing the digestion period.

Recommended Viscosity Procedures

In the 1933 Subcommittee Report (Bayfield, 1933), one method for determining the viscosity of acidulated flour-water suspensions was outlined as a tentative procedure. Since that time a large amount of work has been done, and it now seems advisable that more than one method

be recommended in view of the multiplicity of uses to which the viscosity test may be used. The method outlined tentatively in 1933 now also requires revision owing to several improvements having been made to the method as laid down at that time.

STANDARD EQUIPMENT.—MacMichael viscosimeter, using a No. 30 wire, flat disk bob, speed 12 r.p.m. The disk bob is to be so adjusted that the bottom of the bob is 0.250 ± 0.005 inches from the bottom of the viscosimeter bowl. A micrometer depth gauge, reading in thousandths of an inch, is to be used in making this adjustment. Before making any determinations the viscosimeter is to be checked for the zero point, machine must be level so that the bob turns freely, and the speed should be carefully regulated.

LACTIC ACID.—It is recommended that a stock solution of normal lactic acid be prepared according to the method outlined by Reiman (1934).

PREPARATION OF FLOUR SUSPENSION.—The weighed amount of flour is placed in a dry 500 cc. Erlenmeyer flask. (The flour, as well as other materials and equipment, should have been previously warmed to 30° C.) Pour 100 cc. of distilled water into the flask, tightly stopper, and shake vigorously (180 to 200 shakes per minute) for 1 minute. Remove the stopper, add 3 to 4 drops of caprylic alcohol, re-stopper, and give 10 additional shakes. The suspension is now ready for pouring into the viscosimeter bowl. The flask is held horizontally with one or two fingers over the rubber stopper during the shaking of the suspension. The upper part of the arm is held rather rigidly and the shaking motion is obtained by moving the flask to and from the body, largely by action of the wrist and elbow.

THE VISCOSITY DETERMINATION.—The suspension after preparation is immediately poured into the bowl of the viscosimeter. The contents of the bowl are then stirred by agitating the suspension with the bob, using an up and down, back and forward, circular motion. (The amount and time of stirring influence the viscosity reading and each stirring should consist of 25 of the circular motions, requiring 11 seconds.) The bob is now carefully suspended and the reading is taken after dampening the swings by placing a finger on the indicator pointer and then gently touching the swinging dial.

The second reading is made after adding 1 cc. of normal lactic acid. The third (and subsequent readings) is taken after adding 2 cc. of the same acid. A 1 and a 2 cc. pipette should be used for adding these increments of lactic acid. Usually a total of 7 cc. is sufficient, but the 2 cc. increments should be added and readings taken until either no further increase or a decrease in viscosity results from the added acid.

The second and subsequent readings are obtained in the following manner: After stopping the motor, the bob is detached with the right hand and used for stirring the suspension during and after adding the lactic acid, which is added by means of a pipette held in the left hand. The 25 stirs are commenced as soon as the lactic acid starts to run from the pipette. Upon completion of stirring the bob is re-suspended, the motor is started, and the reading is taken as previously outlined. This described procedure may be referred to as the "several increment method"; *i.e.*, several increments of normal lactic acid are used instead of adding all the acid at one time.

SUGGESTED PROCEDURES.—The following procedures all employ the above basic method for making the suspension and for obtaining the viscosity readings. Upon the information desired will depend the choice of procedure to be used.

(1) To be used for research purposes or refined work when it is desired to determine the strength of a flour. Twenty grams of flour on a 15% moisture basis are to be used in making the suspension. The suspension is allowed to digest for 1 hour at 30° C. before taking the viscosity readings. The contents of the flask are agitated by rotating the flask after 30 minutes digestion and again immediately prior to pouring into the viscosimeter bowl.

(2) Quick method for routine laboratory control work with typical soft wheat flours only. This method is to give an idea of the characteristics of a flour as it is received. The method is the same as in (1), excepting that no digestion period is employed.

(3) As a tentative measure for protein quality it is proposed to use sufficient flour to equal 2 g. of protein, not corrected for moisture basis, in the suspension; otherwise, the method is the same as in (1). It is suggested that additional investigations be carried out concerning the possibilities for determining protein quality.

Conclusions

Collaborative and other results obtained by members of this subcommittee indicate that the tentative method published in 1933 should be revised.

The subcommittee now believes that more than one viscosity procedure should be recommended, although it must be pointed out that results obtained by one procedure may not be translated into terms of another.

Two methods are presented for determining the strength and general characteristics of flours.

One method for determining protein quality is presented in tentative

form, with the recommendation that further work be done with this test before drawing a final conclusion regarding its possibilities.

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The Subcommittee on Viscosity is essentially a working committee and the chairman wishes to express his appreciation to the members—G. L. Alexander, R. M. Bohn, R. S. Gilmer, W. E. Brownlee, Chester Evans, George Garnatz, Walter Reiman, C. G. Harrel, L. A. Mennel, C. F. Evert, C. O. Oppen, H. W. Putnam, V. Shiple, H. M. Simmons, B. Sullivan, and E. L. Ulrey—who have loyally cooperated and made possible the recommendations presented herein.

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EFFECT OF PRESSURE ON RATE OF GAS PRODUCTION IN YEAST FERMENTATION [1]

R. M. SANDSTEDT and M. J. BLISH

Department of Agricultural Chemistry, University of Nebraska, Lincoln, Nebraska

(Read at the Annual Meeting, June 1936)

It has occasionally been suggested that pressure is a factor that may seriously influence rate of gas production in yeast fermentation, and that pressure should therefore be kept constant in methods that involve the quantitative measurement of rate of gas production.

Sandstedt and Blish [2] have proposed for the measurement of the gassing power of flour a manometric procedure in which rate of gas production is measured in terms of pressures recorded at specified time intervals.

During investigations involving dough fermentation with bakers' compressed yeast over very long time periods we have found no evidence that pressure variations from atmospheric pressure up to several hundred additional millimeters of mercury pressure significantly influence the normal rate of gas production. In fact, we feel justified in concluding that pressure variations over this range do not affect rate of gas production.

This conclusion is based upon results noted when dough fermentation in the "pressuremeter" is allowed to proceed for such a long period of time that it becomes necessary to release the pressure several times during the process in order to prevent the mercury from being blown out through the top of the manometer. When the total accumulated pressure values are plotted against time, it has invariably been found that a sudden release of pressure fails to produce any perceptible break in the continuity and slope of the curve, which becomes essentially a straight line after a certain stage of the fermentation process is reached. This is typically illustrated in Figure 1, in which the curves represent prolonged "gassing power" tests conducted according to the method described by Sandstedt and Blish.[2] During the course of the time interval shown on the graph, pressures were released two or three times.

[1] Published with the approval of the Director as Paper No. 181; Journal Series, Nebraska Agricultural Experiment Station.

[2] Sandstedt, R. M., and Blish, M. J. Yeast variability and its control in flour gassing power tests. Cereal Chem. **11**: 368–383 (1934).

From the nature of the curves in Figure 1, it is evident that rate of fermentation was not significantly influenced by releasing the pressures several times during the course of the fermentation period. This justi-

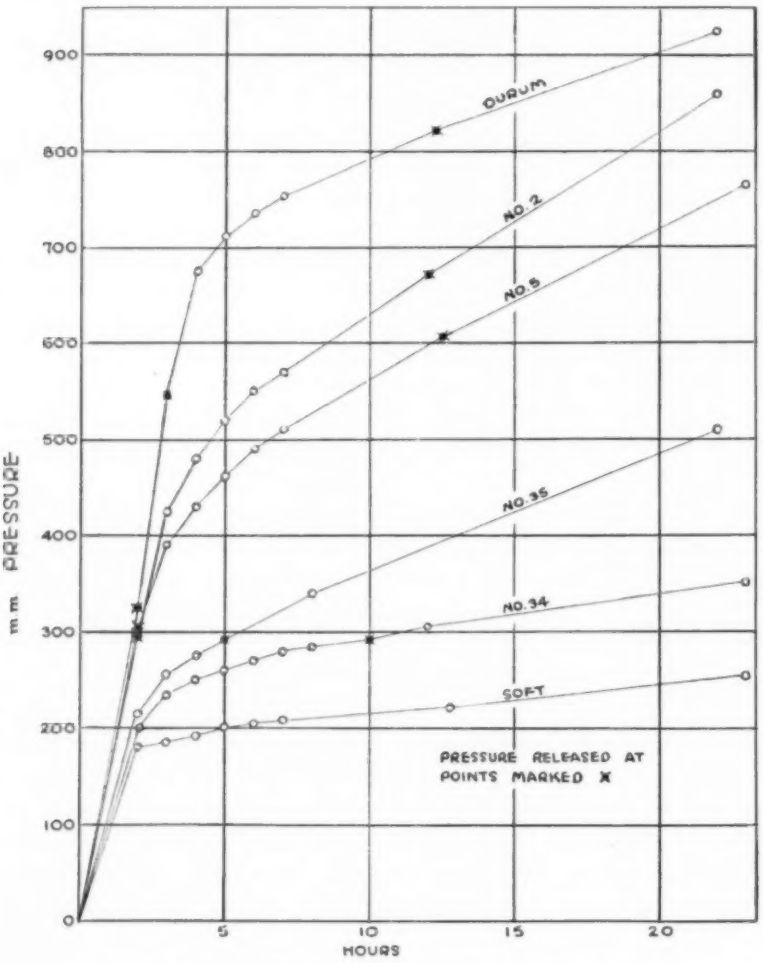

Fig. 1. Manometric determination of gassing power.

fies the conclusion that, over a fairly wide range at least, pressure is not a serious factor as a regulator of yeast fermentation.

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